



The University Of Sheffield.



Global warming: Carbon-nutrient interactions and warming effects on soil carbon dynamics

Submitted by

ANCUTA ASANDEI

to the University of Exeter as a thesis for the degree of

Doctor of Philosophy in Geography

September 2014

This thesis is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature

ABSTRACT

In order to predict how terrestrial ecosystems will respond to global change, there is growing recognition that we need to better understand linkages between plant and soil processes. Previously the factors and processes with potential to influence the terrestrial carbon (C) cycle have been investigated in isolation from each other. This study investigated the interactions of nutrient availability and warming in controlling the soil carbon dynamics, with regards to the fate of already sequestered carbon in soil, under conditions of increasing atmospheric temperatures. The project objectives were addressed by three independent experiments designed to explain specific components of the carbon-nutrient cycle interactions, and the findings brought together to describe the implications for future soil carbon storage.

The main measurements collected throughout this project included soil carbon dioxide (CO₂) fluxes, partitioned into autotrophic and heterotrophic components, net ecosystem exchange and respiration fluxes, and background soil moisture and temperature data, backed by gas, soil and biomass analyses. In the two field experiments, these measurements were taken from plots with or without any inorganic nutrient additions or in the presence or absence of legumes providing biological nitrogen addition to the ecosystem. In the laboratory, temperature and nutrient availability were manipulated within the ecosystem.

The reduction in decomposition rates, without reduction of productivity as a result of inorganic nutrient additions, indicated the potential for increasing C storage. There was also evidence that nutrient availability controls the strength of the link between plant and soil processes in semi-natural grasslands. The yields, decomposition rates and soil C fluxes recorded in the presence and absence of legumes provided some evidence of N₂ fixation, improving ecosystem productivity and soil properties while reducing soil C effluxes, in a managed grassland. In the laboratory, the warming of soils from lysimeters with and without plants, receiving or not receiving fertiliser, supported the findings from field experiments regarding the importance of the soil-plant link in controlling C fluxes. However, C stocks and $\delta^{13}\text{C}$ analyses showed that over a year's worth of warming and nutrient manipulations made little difference to the amount of C stored in the soil, indicating that edaphic factors have greater control over the response of C dynamics to increased temperatures.

ACKNOWLEDGEMENTS

“If we knew what it was we were doing, it would not be called research, would it?”

Albert Einstein

Just like Albert Einstein suggested, I started this PhD not knowing what I was doing, and during the last four years I did a lot of ‘research’ trying to find out. This journey started at a difficult time in my life when I was struggling to find a place for myself in the world, but the most unexpected thing gave me new hope: a letter! That simple act of opening an envelope propelled me into a wonderful new world, of Science! I can only begin to thank my supervisors for the great opportunity they gave me and for the experiences along the way.

It was a privilege to have met and worked alongside so many great minds, from which I have learned so much. I would like to thank Dr. Iain Hartley and Prof. David Hopkins for giving me a piece of their knowledge and I only wish I had more opportunities to spend in their presence. Their constant support, encouragements and constructive critique brought me here today, about to evolve into the next stage of my career. Special thanks go to Iain who was always there from the beginning, to guide me through the hard and good times and hopefully can gauge some progress....finally. My only hope is that I have not disappointed! Thank you for the prompt feedbacks, for constantly helping me move forward and for teaching me how to write like a scientist. Many thanks to my second supervisor at the university, Prof. Timothy Quine for making time to give guidance, support and for always finding solutions to my requests.

My gratitude goes to everyone at the James Hutton Institute, where it all started, for providing the facilities, co-operation and funding to support my research. Special thanks to Dr. Tim Daniell for stepping up as a supervisor when it was necessary, for accommodating my visits to Scotland and introducing me to some very interesting scientists.

Research takes you to so many unexpected places and even if I did not travel abroad for work, I had the chance to visit some unique place in the UK and meet

wonderful new people. That is why I would like to thank Dr. Victoria Sloan and Dr. Gareth Phoenix for allowing me to take advantage of their experiments in the Peak District and for welcoming me at the University of Sheffield. Thanks to Victoria I was always on time, fed and entertained in the field; the time I spent in your company was relaxing and enriching at the same time; thank you for being my lovely assistant!

Another unexpected chance took me close to Dartmoor at North Wyke Research Centre. Many thanks to my supervisors at the farm, Dr Jennifer Dungait, Dr. Robert Dunn and Dr. Deborah Beaumont for the patience in dealing with an avalanche of questions and requests and for insights into the life of an agricultural scientist. To everyone working there, and I think I met everyone, thank you for welcoming me into you family!

To the army of people at the University of Exeter, my home for three years, I thank you deeply. Without each and everyone's influence I would have been poor, sad and lonely. To my colleges and friends, I will always remember you!

	PAGE
ABSTRACT	2
ACKNOWLEDGEMENTS	3
TABLE OF CONTENTS	4
LIST OF FIGURES	9
LIST OF TABLES	14
LIST OF PICTURES	15
LIST OF ABBREVIATIONS	16
1. CHAPTER I – GENERAL INTRODUCTION AND RESEARCH QUESTIONS	19
1.1. ANTHROPOGENIC IMPACTS ON TERRESTRIAL ECOSYSTEMS	19
1.1.1. Climate change	19
1.1.2. Nitrogen deposition	22
1.1.3. Summary	25
1.2. CARBON AND NUTRIENT CYCLING IN TERRESTRIAL ECOSYSTEMS	26
1.2.1. Key carbon cycle processes	26
1.2.1.1. <i>Photosynthesis and net primary productivity</i>	29
1.2.1.2. <i>Soil respiration</i>	30
1.2.1.3. <i>Summary</i>	31
1.2.2. Nutrient cycling focusing N and P	32
1.2.2.1. <i>Key nitrogen cycle processes</i>	32
1.2.2.2. <i>Key phosphorus cycle processes</i>	37
1.2.3. Interactions between carbon and nutrient cycling	39
1.2.3.1. <i>Links between decomposition and plant growth</i>	40
1.2.3.2. <i>Mycorrhizal fungi</i>	42
1.2.3.3. <i>Summary</i>	43
1.3. NUTRIENT AND CARBON CYCLING IN THE CONTEXT OF GLOBAL CHANGE	44
1.3.1. Elevated CO ₂	44
1.3.2. Climate Change	45
1.3.3. Grassland management for carbon sequestration	49
1.3.4. Carbon, nutrients and warming	52
1.3.5. Summary	53
1.4. RESEARCH REQUIREMENTS AND KEY OPEN QUESTIONS	55
1.4.1. Nutrient availability and ecosystem carbon storage	55
1.4.2. Nitrogen fixation and carbon uptake	55
1.4.3. Nutrient availability controls over ecosystem responses to warming	56
1.5. MAIN RESEARCH AIMS	57
2. CHAPTER II - THE IMPACTS OF NUTRIENT ADDITION ON DECOMPOSITION RATES AND THE LINKS BETWEEN PLANT AND SOIL PROCESSES IN TWO SEMI-NATURAL GRASSLANDS	59
2.1. INTRODUCTION	59
2.1.1. Research questions	59
2.1.2. Current perspective	62
2.1.3. Research requirements	66

2.2.	WORK AND SITE JUSTIFICATION	67
2.3.	AIMS AND OBJECTIVES	69
2.4.	HYPOTHESES	69
2.5.	METHODOLOGY	70
2.5.1.	Site description	70
2.5.2.	Experimental design and data collection	73
2.5.2.1.	<i>Soil temperature and moisture measurements</i>	73
2.5.2.2.	<i>Soil respiration measurements</i>	73
2.5.2.3.	<i>Net ecosystem CO₂ exchange</i>	77
2.5.3.	Calculations and statistical analysis	79
2.6.	RESULTS	81
2.6.1.	Acidic grassland	81
2.6.1.1.	<i>Soil temperature and moisture</i>	81
2.6.1.2.	<i>Soil CO₂ fluxes</i>	81
2.6.1.3.	<i>Heterotrophic and autotrophic respiration</i>	84
2.6.1.4.	<i>Ecosystem productivity</i>	87
2.6.2.	Calcareous grassland	90
2.6.2.1.	<i>Soil temperature and moisture</i>	90
2.6.2.2.	<i>Soil CO₂ fluxes</i>	90
2.6.2.3.	<i>Heterotrophic and autotrophic respiration</i>	91
2.7.	DISCUSSION	93
2.7.1.	Soil carbon fluxes and productivity in an acidic semi-natural grassland	93
2.7.2.	Soil carbon fluxes in a calcareous semi-natural grassland	95
2.7.3.	Stochastic nutrient additions	96
2.8.	CONCLUSIONS	97
3.	CHAPTER III – THE EFFECT OF NITROGEN FIXATION ON DECOMPOSITION RATES AND CARBON STORAGE IN A MANAGED GRASSLAND	99
3.1.	INTRODUCTION	99
3.1.1.	Research questions	99
3.1.2.	Current perspective	101
3.1.3.	Research requirements	104
3.2.	WORK AND SITE JUSTIFICATION	106
3.3.	AIMS AND OBJECTIVES	107
3.4.	HYPOTHESES	107
3.5.	METHODOLOGY	108
3.5.1.	Site description	108
3.5.2.	Experimental design and data collection	111
3.5.2.1.	<i>Soil respiration measurements</i>	111
3.5.2.2.	<i>Soil temperature and moisture measurements</i>	111
3.5.2.3.	<i>Litter bags</i>	114
3.5.2.4.	<i>Soil Cores</i>	115
3.5.2.5.	<i>Biophysical measurements made by NW team</i>	115
3.5.3.	Calculations and statistical analysis	117

3.6.	RESULTS	118
3.6.1.	Soil temperature and moisture	118
3.6.2.	Soil CO ₂ fluxes	118
3.6.3.	Heterotrophic and mycorrhizal respiration	120
3.6.4.	<i>In-situ</i> litter decomposition	121
3.6.5.	Soil physical and chemical characteristics	123
3.6.5.1.	<i>Moisture, compaction and bulk density</i>	123
3.6.5.2.	<i>Soil C, N, P and pH</i>	123
3.6.6.	Vegetation cover, diversity and yield	125
3.6.7.	Soil nutrient loss	127
3.7.	DISCUSSION	128
3.7.1.	Changes in soil physical and chemical characteristics	129
3.7.2.	Ecosystem productivity, decomposition and CO ₂ efflux	130
3.8.	CONCLUSIONS	133
4.	CHAPTER IV - THE IMPACT OF NUTRIENT ADDITIONS, WARMING AND CLIPPING ON ECOSYSTEM PRODUCTIVITY AND CARBON STORAGE	135
4.1.	INTRODUCTION	135
4.1.1.	Research questions	135
4.1.2.	Current perspective	136
4.1.2.1.	<i>The need for linking plant and soil measurements</i>	137
4.1.2.2.	<i>The role of C₃ and C₄ in carbon cycle research</i>	138
4.1.3.	Summary	139
4.2.	WORK AND SITE JUSTIFICATION	140
4.3.	AIMS AND OBJECTIVES	141
4.4.	HYPOTHESES	142
4.5.	METHODOLOGY	143
4.5.1.	Site and experimental design	143
4.5.1.1.	<i>Lysimeters and warming system</i>	144
4.5.1.2.	<i>Frame and lights</i>	144
4.5.1.3.	<i>Soil and seeds</i>	145
4.5.1.4.	<i>Water addition regime</i>	147
4.5.2.	Treatment manipulations	147
4.5.2.1.	<i>Nutrient additions</i>	148
4.5.2.2.	<i>Warming</i>	148
4.5.2.3.	<i>Clipping</i>	149
4.5.2.4.	<i>Soil only lysimeters</i>	149
4.5.2.5.	<i>Pesticide addition</i>	151
4.5.3.	Data collection and analysis	153
4.5.3.1.	<i>Soil temperature and moisture measurements</i>	153
4.5.3.2.	<i>CO₂ flux measurements</i>	154
4.5.3.3.	<i>Leachate collection and analysis</i>	156
4.5.3.4.	<i>Soil, gas and vegetation sampling and analysis</i>	157
4.5.4.	Calculations and statistical analysis	159

4.6.	RESULTS	160
4.6.1.	Baseline measurements	160
4.6.1.1.	<i>Soil temperature and moisture</i>	160
4.6.1.2.	<i>Leaching rates</i>	163
4.6.2.	Ecosystem productivity and parameter variations	164
4.6.2.1.	<i>Ecosystem respiration (ER)</i>	164
4.6.2.2.	<i>Net ecosystem exchange (NEE)</i>	167
4.6.2.3.	<i>Gross primary productivity (GPP)</i>	169
4.6.2.4.	<i>Diurnal variation of ecosystem parameters</i>	171
4.6.3.	Isotopic analyses	174
4.6.3.1.	<i>Gas samples</i>	174
4.6.3.2.	<i>Soil samples</i>	174
4.6.4.	Total carbon content and carbon stocks	177
4.6.5.	Rates of nutrients loss	179
4.6.5.1.	<i>Nitrogen (N) loss rates</i>	179
4.6.5.2.	<i>Phosphorus (P) loss rates</i>	182
4.6.5.3.	<i>Cations loss rates</i>	184
4.7.	DISCUSSION	185
4.7.1.	Ecosystem carbon fluxes	185
4.7.1.1.	<i>Effects of nutrient availability on carbon fluxes</i>	185
4.7.1.2.	<i>Warming impacts on carbon fluxes</i>	185
4.7.1.3.	<i>Clipping influence over the soil carbon fluxes</i>	186
4.7.1.4.	<i>Main and interactive effects of treatment on carbon fluxes</i>	187
4.7.2.	Changes in respired CO ₂ source	187
4.7.3.	Ecosystem properties and productivity	188
4.7.3.1.	<i>Soil and vegetation physical and chemical characteristics (biomass yields, SOM, N, P)</i>	188
4.7.3.2.	<i>Loss of nutrients from the ecosystem</i>	188
4.7.3.3.	<i>Changes in soil C stocks</i>	189
4.8.	CONCLUSIONS	190
5.	CHAPTER V - FROM EXPERIMENTAL HALL, TO THE FIELD AND THE WORLD: NUTRIENT ENRICHMENT AND WARMING CONSEQUENCES FOR SOIL CARBON STORAGE	191
5.1.	INTRODUCTION	191
5.2.	NUTRIENT AVAILABILITY AND DECOMPOSITION RATES	195
5.2.1.	Reasons for difference between Wardlow and North Wyke	196
5.2.2.	Explanations for Wardlow findings: the direct effect of nutrient availability on decomposition	198
5.2.3.	Explanations for Wardlow findings: The priming effect control over decomposition rates	195
5.3.	TEMPERATURE AND NUTRIENT INTERACTIONS CONTROLLING SOIL CARBON	200
5.4.	FUTURE CARBON SEQUESTRATION IN SOIL	202
5.5.	LIMITATIONS	203
5.6.	CONCLUSIONS	204

6.	APPENDICES	205
6.1.	APPENDIX A	206
6.2.	APPENDIX B	211
6.3.	APPENDIX C	241
7.	REFERENCES	251

LIST OF FIGURES	PAGE
Figure 1.1. Contrasts between observed and simulated climate changes in land surface temperatures (yellow panels), Arctic and Antarctic sea ice extent (white panels) and upper ocean heat content – OHC (blue panels). All data time series are averaged for each decade starting 1880 for temperatures, 1960 for OHC and 1979 for sea ice. Dashed lines in the land surface temperatures mean that the analysed areas are under 50% and for the OHC and sea ice it represents only adequate data coverage compared to the higher quality of the data represented in solid lines (Stocker et al., 2013)	21
Figure 1.2. Diagram of key terrestrial ecosystem carbon exchange processes. Carbon inputs are indicated by the blue arrow, carbon movement and allocation through the ecosystem is represented by the green arrows and carbon release is indicated by the red arrows	28
Figure 1.3. Schematic representation of the terrestrial nitrogen cycle. Nitrogen in the environment can be present as organic nitrogen, ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), nitric oxide (NO) or inorganic nitrogen gas (N_2). Nitrogen becomes accessible to plants for growth through processes of fixation, mineralization and nitrification. The opposite reactions of denitrification, volatilization, immobilization, and leaching result in permanent or temporary N losses from the root zone	36
Figure 1.4. Schematic representation of the phosphorus cycle, illustrating the succession of processes in the biosphere. There is no gaseous phase in the phosphorus cycle. Phosphorus is released by the weathering of rocks and minerals or from phosphorus-rich deposits formed in the ocean and migrated to the land as sediments. Then it is taken up by plants where is transformed into organic compounds. After the plants decay the phosphorus is returned to the soil, where a large part is transformed into insoluble compounds, while a small part can be lost by runoff to the ocean	38
Figure 1.5. Schematic representation of carbon and nutrient cycle interactions, illustrating the interdependence of their key processes and the potential for ecosystem carbon sequestration	39
Figure 1.6. A schematic representation of the soil organic matter (SOM) dynamic and soil inorganic C (SIC), with measurable pools, in relation to their quality and availability (modified from Six <i>et al.</i> (2000)	48
Figure 1.7. UK map illustrating the natural and man-made habitats, emphasizing the extent of the improved grasslands in comparison with those unimproved (source: NERC website)	51
Figure 2.1. UK maps illustrating the distribution of semi-natural grasslands (A.) and areas of total oxidised N deposition (B.); highlighted in red and black are the experiments site under investigation in this chapter, at Wardlow Hay Cop in Peak District National Park (modified from CEH & DEFRA).	64
Figure 2.2. Collar position in each of the two grasslands; the hashed side of each plot represents the continuation of nutrient treatments and the blank side characterising the recovery half. Each of the blocks: A, B, C represent the three replicates for the six nutrient additions	72
Figure 2.3. Set of collar types and insertion depth for each of the grasslands (a. – acidic grassland; b. – calcareous grassland). <i>RMS</i> - shallow collars measuring all soil respiration flux components: roots (thick lines), mycorrhizal hyphae (thin lines) and soil heterotrophs (grainy pattern); <i>MS</i> - deep collars with three	76

windows covered with 20 μ m mesh, severing and excluding the roots and allowing in-growth of mycorrhizal hyphae; *S* - deep collars with three windows covered with 1 μ m mesh excluding both roots and mycorrhizal hyphal in-growth. The function of the *RMS* and *S* collars in the calcareous grassland is the same as the acidic one; the *S* collar is missing the mesh windows because its integrity would have been compromised on insertion and contact with the irregular bedrock in this grassland

- Figure 2.4.** Soil respiration rates. CO₂ fluxes recorded for each collar type: *RMS*, *MS*, and *S*, in May (a, e), August (b, f), early September (c, g) and late September (d, h). Data are presented by nutrient treatment (with and without P present). Error bars are \pm SE (n=3) **83**
- Figure 2.5.** Heterotrophic and autotrophic respiration rates in the acidic grassland. Fluxes are grouped by the presence of nitrogen (N) in the applied treatment and indicate the respirations rates calculated for the plots without P present (light bars) and with P additions (dark bars). Error bars are \pm 1SE (n=3) **85**
- Figure 2.6.** Relative contribution of root and arbuscular mycorrhizal fungi (black bars) and soil (red bars) respiration. Bars represent the total percentage of below-ground respiration in May (a), August (b), early September (c), and late September (d) **86**
- Figure 2.7.** Light curves measured in the acidic grassland, on four of the investigated treatment plots (C, P, HN & HNP) in August (a) and September (b). Error bars are \pm 1SE (n=3). The parameters used in fitting these lines are shown in Table 2.2 **87**
- Figure 2.8.** Gross primary productivity (GPP) and ecosystem respiration (ER) calculated from the net ecosystem CO₂ exchange data collected in August (a) and September (b). Error bars are presented as \pm 1SE (n=3) **89**
- Figure 2.9.** Respiration rates in the calcareous grassland, recorded for both collar types (*RMS* and *S*). Data are shown separately for the treatment receiving different levels of N only (a, b, c) and those who previously received P treatment (e, f, g), but has ceased after a couple of years after initiation. The graphs illustrate measurements taken in May (a, d), August (b, e) and September (c, f). Error bars are presented as \pm 1SE (n=3) **92**
- Figure 3.1.** Experimental design of a replicate block. Thick dashed line separates grazed from cut plots (by stock proof fencing); thick dotted line separates Typical (T) managed plots from Rested (R) plots (by temporary stock proof fencing); thin dashed line distinguishes plough (P) from minimal cultivation (MC) plots; the undisturbed existing grass plots in the grass only swards are indicated by T E† (no fencing between T plots). The red blocks represent the plots chosen for the experiments carried out for the purpose of this chapter **109**
- Figure 3.2.** WEB experiment plots, all 72, are represented in light green and grey border. The dark green plots bordered in brown are the plots used for the experiments described in this chapter. The numbers indicate the plot ID (1 to 72), while the letters represent the sward ID (G= grass mix; GL= grass + legume mix; GLF= grass + legume + forb mix) **110**
- Figure 3.3.** Set of collar types and insertion depths. *RMS* - shallow collars measuring all soil respiration flux components: roots (thick lines), mycorrhizal hyphae (thin lines) and soil heterotrophs (grainy pattern); *S* - deep collars excluding both roots and mycorrhizal hyphal in-growth (modified from Heinemeyer *et al.* (2007) **113**
- Figure 3.4.** Example of sub-plot layout and the two types of collars installed **113**

Figure 3.5.	Sub-plot layout, including each of the litter bags and the due collection time point	114
Figure 3.6.	Soil CO ₂ fluxes recorded in September 2012 (a) and March 2013 (b) for both treatments (G & GLF) at the RMS and S collars. Error bars are $\pm 1SE$ (n=3)	119
Figure 3.7.	Mean weight loss (%) for each litter type (a – grass, b – wood), collected at four time points in the year (September, November, January and March). Error bars are $\pm SE$ (n=3)	122
Figure 3.8.	Mean soil C, N, P and pH ($\pm SE$) in the first year of the experiment 2008, and the last two; 2011 and 2012. Error bars are $\pm SE$ (n=3)	124
Figure 3.9.	Mean legume, forb and all grass cover (%/ m ²) for each year of the experiment (2009 to 2012) in the G and GLF treatment plots	126
Figure 3.10.	Mean yearly herbage yield calculated for the typical cut plots under grass and grass-legume-forb sward. Error bars are $\pm SE$ (n=3)	126
Figure 3.11.	Mean total oxidized N (a) and mean total P (b), measured in water leachate collected from 2009 to 2012, for the original grassland plots, grass plots (G) and grass-legume-forb plots (GLF). Error bars are $\pm SE$ (n=3)	128
Figure 4.1.	Schematic diagram of the lysimeter design, illustrating components, dimensions and layers: the side view of a lysimeter (a), the tubing system staggered on two levels inside each lysimeter (b) and the soil profile layers (c – 100% soil; d – 20:80% soil + sand mix; e – 100% sand and f – gravel).	146
Figure 4.2.	The timeline of the experiment, illustrating the start of each treatment applied	147
Figure 4.3.	Temperature difference between ambient and warmed lysimeters (approx. 5°C) sampled from data collected using temperature loggers. The purple line (planted lysimeter – e.g. WHU) and pale orange line (soil only lysimeter – e.g. WH) show that warming was applied, and the green line (planted lysimeter – e.g. AHU) and the pale blue line (soil only lysimeter – e.g. AH) represent the lysimeters at ambient temperature	148
Figure 4.4.	Schematic representation ‘Pots’ experiment layout and treatment assignment in each block (A, B, C, D). The circles with a green centre represent the 32 pots where plants were grown and the circle with a brown centre are the 16 pots containing bare soil only. The red colour around the circumference indicates the pots were warmed approximately 5°C above ambient temperatures, represented by the blue circumferences	152
Figure 4.5.	Soil moisture decline from the watering day (14.01.2013) until two days later (16.01.2013)	153
Figure 4.6.	Schematic diagram of the sampling loop used to extract the gas samples to be analysed by $\delta^{13}C$	158
Figure 4.7.	Temperature changes and differences between four treatments before and after the warming was applied. The stars (*) indicate the dates coinciding with the CO ₂ efflux measurements.	161
Figure 4.8.	Soil moisture timelines for the soil only pots (a.), unclipped planted pots (b.) and clipped planted pots (c.). Comparison is made between the pots with and without added nutrients and between control and warmed pots. These can be observed relative to the three main manipulations: nutrient additions (continuous vertical line), warming (dashed vertical line) and clipping (dotted vertical line). Error bars are $\pm SE$ (n=4)	162

Figure 4.9.	Monthly cumulative leaching rates from the soil only lysimeters (a), the unclipped lysimeters (b), and the clipped lysimeters (c).	163
Figure 4.10.	Ecosystem respiration timeline for soil only pots (a.), unclipped planted pots (b.) and clipped planted pots (c.), relative to the main manipulations applied, warming (dashed vertical line) and clipping (dotted vertical lines). Error bars are \pm SE (n=4)	166
Figure 4.11.	Net ecosystem exchange timeline for soil only pots (a.), unclipped planted pots (b.) and clipped planted pots (c.), relative to the main manipulations applied, warming (dashed vertical line) and clipping (dotted vertical lines). Error bars are \pm SE (n=4)	168
Figure 4.12.	Gross primary productivity timeline for soil only pots (a.), unclipped planted pots (b.) and clipped planted pots (c.), relative to the main manipulations applied, warming (dashed vertical line) and clipping (dotted vertical lines). Error bars are \pm SE (n=4)	170
Figure 4.13.	Diurnal variation of the ecosystem fluxes in the soil only lysimeters, in June – July 2012 before any manipulations were applied to the lysimeters (a.) and in August 2012 after fertilisation treatment began (b.). Both panels of the graph represent data before warming started in December 2012; the variation in soil temperature, illustrated by the green line, is due to the changes in atmospheric temperature between night and day. The break in the line from panel (a) represents a power cut, thus data could not be used. The sudden shift in the lines is represented by the lights coming on and warming up the air and soil in the lysimeters. The lights were set to switch on at 6 am and switch off at 19 pm	172
Figure 4.14.	Diurnal variations of ecosystem fluxes relative to the soil temperatures recorded in the control (blue lines) and warmed (red lines) lysimeters. The fluxes illustrated represent comparisons between low and high nutrient additions (ALU-WLU & AHU-WHU) in ambient and warmed lysimeters (a.) and between clipped and unclipped (ALC-WLC & ALU&WLU) ambient and warmed lysimeters receiving low nutrient (b.) The dip in the temperature from the warmed lysimeters, was due to technical issues with the thermostat.	173
Figure 4.15.	Gas sample results for the $\delta^{13}\text{C}$ analysis indicating the levels recorded in May 2013 (a.) and August 2013 (b.). Error bars are \pm SE (n=4)	175
Figure 4.16.	Soil sample results for the $\delta^{13}\text{C}$ analysis indicating the levels in the soil only (a.), clipped (b.) and unclipped (c.) planted pots, 15 months after the initial soil was tested. Error bars are \pm SE (n=4)	176
Figure 4.17.	Soil carbon stock in the pots after 15 months of experiments compared to the initial soil carbon stock. Error bars are \pm SE (n=4)	178
Figure 4.18.	Total carbon content of the above ground biomass at the end of the experiment - 15 months of growing. Error bars are \pm SE (n=4)	179
Figure 4.19.	Cumulative rates of nitrogen in leachate, relative to the main treatments applied to the pots: nutrient additions (continuous vertical line), warming (dashed vertical line) and clipping (dotted vertical line). Pots were grouped by manipulations, as soil only pots (a.), unclipped planted pots (b.) and clipped planted pots (c.). Error bars are \pm SE (n=4)	181
Figure 4.20.	Cumulative rates of phosphorus loss in leachate, relative to the main treatments applied to the pots: nutrient additions (continuous vertical line), warming (dashed vertical line) and clipping (dotted vertical line). Pots were grouped by manipulations, as soil only pots (a.), unclipped planted pots (b.) and clipped planted pots (c.). Error bars are \pm SE (n=4)	183

LIST OF TABLES	PAGE
Table 2.1. The rate of respiration calculated for the autotrophic components: roots and mycorrhizal fungi and the heterotrophic component during all four sampling sessions. Autotrophic respiration was reported as the sum of root and EM, due to some negative EM values	84
Table 2.2. The parameters used in fitting the lines in Figure 2.7, for all four treatments investigated (C, P, HN, HNP) during the sampling sessions in August and September.	89
Table 3.1. Summary of <i>p</i> values obtained for temperature, moisture and CO ₂ flux by 1-way ANOVA, in September 2012 and March 2013; values with (*) are statistically different	119
Table 3.2. Summary of the C/N ratios of the G and GLF plots, in 2008, 2011 and 2012	125
Table 3.3. Typical cut plots (G and GLF) herbage yield, reported as tonnes per ha and the calculated standard error, for all four years measured (2009 to 2012). Differences in yield between the two seed mixtures (GLF-G) and percentage GLF yield is higher than G yield	126
Table 4.1. Factorial experimental design, illustrating all 12 treatments (a - l) with three factors: each having two levels (nutrient and warming: 0 – no nutrient/ control; 1 – high nutrient/ warming), except clipping with three levels (0 – no biomass pots; 1 – unclipped biomass pots; 2 – clipped biomass pots)	143
Table 4.2. Above-ground biomass height before and after the 2 clipping sessions (14.01.2013 and 09.04.2013)	149

LIST OF PICTURES

PAGE

Picture 2.1.	Wardlow Hay Cop experimental site, in the Peak District National Park, illustrating the location of the two semi-natural grasslands: calcareous and acidic (modified from the DEFRA website)	71
Picture 2.2.	Portable IRGA connected to cylindrical Perspex chamber placed on top of a PVC collar recording soil CO ₂ flux measurements	78
Picture 2.3.	Net ecosystem CO ₂ exchange measurements setup, including the Perspex chamber, battery, IRGA and the PAR Quantum device	78
Picture 4.1.	Illustration of the LI-Cor and lysimeter system used to record ecosystem CO ₂ flux measurements	155

LIST OF ABBREVIATIONS

AM	Arbuscular mycorrhizae
BNF	Biological nitrogen fixation
[CO ₂]	Carbon dioxide concentration
C	Carbon
CH ₄	Methane
CM	Conceptual Model
CO ₂	Carbon dioxide
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
ER	Ecosystem respiration
<i>Eq.</i>	Equation
FACE	Free air CO ₂ enrichment
GHG	Greenhouse gas
GPP	Gross primary production
Gt	Giga-tonne
HNO ₃	Nitric acid
IPCC	Intergovernmental Panel on Climate Change
IRGA	Infrared gas analyser
LU	Livestock units
Mg	Mega-gram
N ₂ O	Nitrous oxide
NEE	Net ecosystem exchange
NH ₃	Ammonia
NH ₄ ⁺	Ammonium
NO ₃ ⁻	Nitrates
NO _x	Nitrogen oxides
NPP	Net primary productivity
N _r	Reactive nitrogen
OHC	Ocean heat content
OM	Organic matter
PAN	Peroxyacetylene nitrate
PAR	Photosynthetically Active Radiation
ppb	Parts per billion
PPFD	Photosynthetic photon flux density
ppm	Parts per million

R _p	Plant respiration
SIC	Soil inorganic matter
SOM	Soil organic matter
Tg	Tera-gram

CHAPTER I

INTRODUCTION

1.1. ANTHROPOGENIC IMPACTS ON TERRESTRIAL ECOSYSTEMS

1.1.1. Climate change

Climate change, as defined in the latest Intergovernmental Panel on Climate Change (IPCC), 2013 report, refers to *'the change in the state of the climate that can be identified by decade-long changes in the mean and variability of its properties'*. Changes in climate over time can be caused by natural variability or as a result of human activity. Anthropogenic activities have resulted in changes to the composition of the atmosphere. Increased concentrations of greenhouse gases (GHG), such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) have the potential to add even more pressure on Earth's systems. The build-up of greenhouse gasses in the atmosphere may cause an increase in global average temperatures by 1.1 – 6.4 °C during the current century (IPCC, 2013). It is highly likely that the current warming is related to CO₂ emissions from burning fossil fuels (which release 8 Gt C y⁻¹) and other human related activities, including land use changes (Houghton, 2001). Evidence of these changes has been compiled from empirical data including observations of increases in air and ocean global average temperatures, widespread melting of snow and ice and rising global average of sea levels (IPCC, 2013).

The IPCC (2007) report, states that due to the increase in atmospheric CO₂ concentration, it is likely (> 66 % probability of occurrence) that in the next 50 to

100 years there will be an additional 2 to 4.5 °C of warming and is very unlikely (< 33 % probability of occurrence) that this warming will be less than 1.5 °C. The most recent IPCC report (IPCC, 2013) emphasises that from 1750 to 2011, CO₂ concentration increased by 40 % from 278 ppm to 390.5 ppm. During the same time interval, CH₄ increased by 150 % from 722 ppb to 1803 ppb, and N₂O by 20 % from 271 ppb to 324.2 ppb in 2011. As one of the most important GHG, CO₂ has registered a growth in annual emissions between 1970 and 2004 by about 80 %, from 5.7 to 10.3 Gt C, which represented 77 % of total anthropogenic GHGs emissions in 2004. Anthropogenic CO₂ emissions to the atmosphere were approximately 555 Gt C between 1750 and 2011; fossil fuel combustion and cement production contributed around 375 Gt C and land use change (including deforestation, afforestation and reforestation) contributed almost 180 Gt C (IPCC, 2013). Data presented in Figure 1.1 demonstrates that global warming is an anthropogenic driven phenomenon. The observed patterns are best explained by models that include combined natural and anthropogenic forcings. However, when the same models are run without anthropogenic forcing, no significant temperature increase is seen. Although no model output will be used in this project, a conceptual model will be proposed in order to illustrate the terrestrial ecosystem processes understanding, that could help predict some of the feedbacks to climate change.

The observed changes in the atmospheric concentration of GHGs are the result of a dynamic balance between anthropogenic emissions and the perturbation of natural processes. The link between natural GHGs emission processes, physical conditions, chemical reactions and biological transformations, within the terrestrial ecosystem, is the key that leads to a partial removal of these gases from the atmosphere by the terrestrial biota. For example, the build-up of CO₂ in the atmosphere is reduced by the terrestrial biota, which take up around 2.8 Gt C y⁻¹ (Arneeth *et al.*, 2010). These interactions between biotic and abiotic factors dictate the way they respond to perturbed atmospheric composition and climate change, leading to the conclusion that the physical climate system and the biogeochemical cycles of these gases are coupled (IPCC, 2013). The need

to better understand the dynamic balance between these processes is a fundamental underpinning for the research described in the thesis.

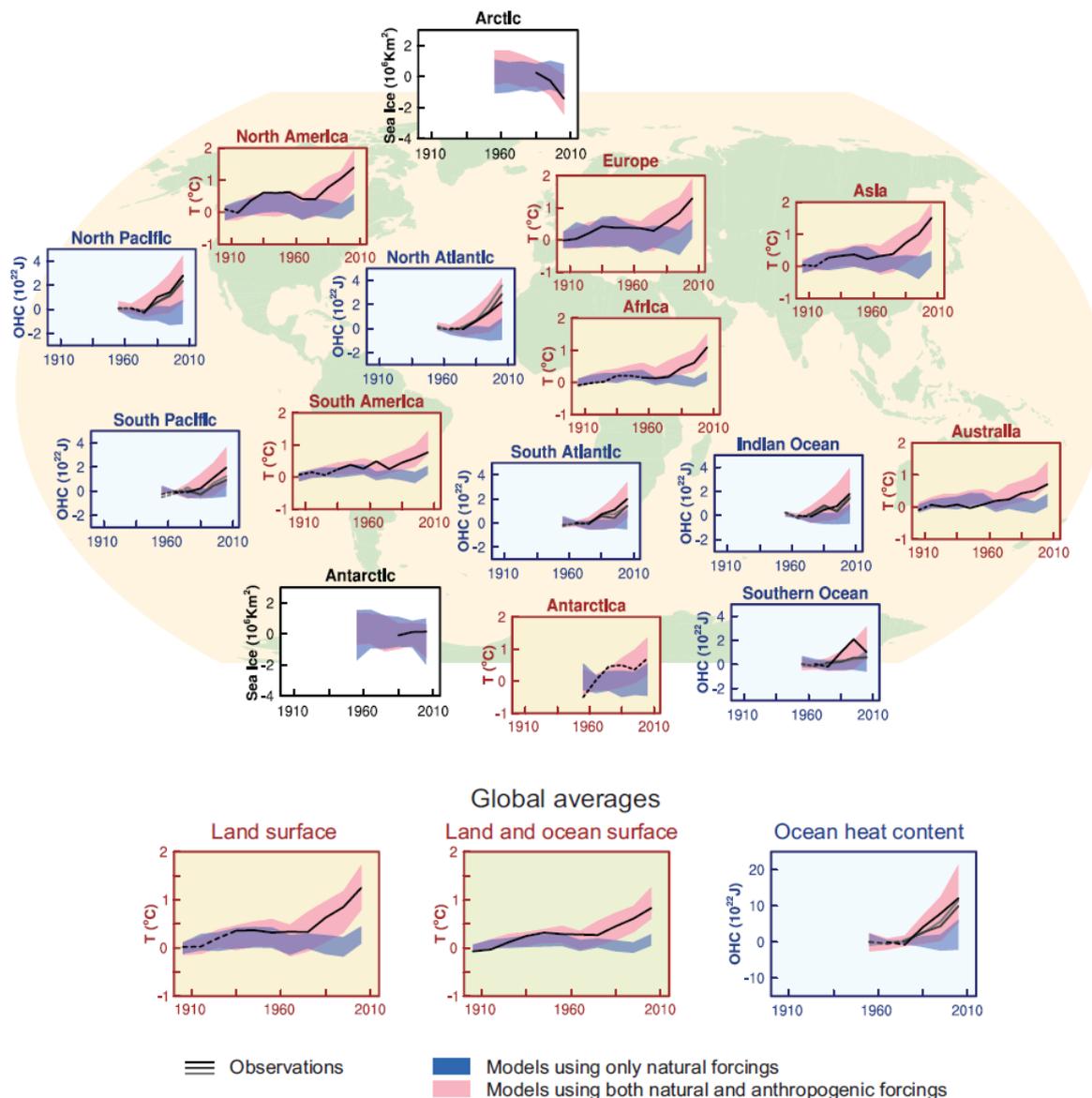


Figure 1.1. Contrasts between observed and simulated climate changes in land surface temperatures (yellow panels), Arctic and Antarctic sea ice extent (white panels) and upper ocean heat content – OHC (blue panels). Models using natural, and natural and anthropogenic forcings are compared, demonstrating that only the inclusion of anthropogenic forcing can explain recent warming trends. All data time series are averaged for each decade, starting with 1880 for temperatures, 1960 for OHC and 1979 for sea ice. Dashed lines in the land surface temperatures mean that the data was collected and analysed for under 50 % of the total areas of land for the OHC and sea ice it represents only adequate data coverage compared to the higher quality of the data represented in solid lines. The shaded bands indicate the 5 to 95 % confidence intervals (IPCC, 2013).

1.1.2. Nitrogen deposition

Nitrogen (N) deposition is defined as the input of reactive nitrogen (N_r) chemical species from the atmosphere to the Earth's surface (IPCC, 2013). This phenomenon causes most concern when assessing its impacts on terrestrial ecosystems. The pollutants that contribute to nitrogen deposition derive mainly from nitrogen oxides (NO_x) and ammonia (NH_3) emissions. In the atmosphere NO_x is transformed to a range of secondary pollutants, including nitric acid (HNO_3), nitrates (NO_3^-) and organic compounds, such as peroxyacetylene nitrate (PAN), while NH_3 is transformed to ammonium (NH_4^+). All these pollutants can be removed from the atmosphere by wet deposition (scavenging of gases and aerosols by precipitation) and by dry deposition (direct turbulent deposition of gases and aerosols) (Fowler *et al.*, 1989). Altogether, N deposition could lead to acidification, fertilisation and eutrophication effects in terrestrial ecosystems.

The severity of the effects, that airborne nitrogen (N) deposition can have on the terrestrial ecosystems, depends on (i) the duration, the total amount, and the N form of the inputs; (ii) the intrinsic sensitivity of the plant species present; and (iii) abiotic conditions in the ecosystem (Bobbink *et al.*, 2010). This information was also used in the development of the concept of 'critical loads', first defined by Nilsson (1988) as '*a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge*'.

Before the Industrial Era, the creation of reactive nitrogen (N_r) from non-reactive atmospheric N_2 occurred primarily through two natural processes: lightning and biological nitrogen fixation (BNF). However, since then, the equilibrium between demand and supply in the terrestrial ecosystems has been broken and N_r is produced now also by human activities and delivered to ecosystems (IPCC, 2013). There are three main anthropogenic sources of N_r : (i) the Haber-Bosch industrial process, used to make NH_3 from N_2 , for N fertilisers and as a feedstock for some industries; (ii) the cultivation of legumes and other crops, which increases BNF; and (iii) the combustion of fossil fuels, which converts

fossil fuel N into nitrogen oxides (NO_x) emitted to the atmosphere and the re-deposited on the ground (IPCC, 2013).

Bobbink and Roelofs (1995), identified the most important impacts of increased atmospheric N deposition upon biological systems as: (i) short-term effects of N gases and aerosols to individual species (critical levels); (ii) soil-mediated effects of acidification; (iii) soil-mediated effects of N enrichment; (iv) increased susceptibility to secondary stress factors (drought, disease, etc.) and, (v) changes in competitive relationships between species, resulting in loss of diversity. Thus, once deposited from the atmosphere, N_r can acidify soils and waters and increase plant productivity in forests, grasslands and freshwaters, which can lead to eutrophication, reduction in biodiversity in terrestrial and aquatic ecosystems and increased nitrate leaching or NO_x fluxes (Sutton *et al.*, 2011, Vitousek *et al.*, 1997). Once in the biosphere, N_r can then be re-emitted to the atmosphere as nitrous oxide contributing to global warming and stratospheric ozone depletion with negative impacts on human health and ecosystem services (Davidson *et al.*, 2012, Galloway *et al.*, 2008).

In a global overview on nutrient management, Sutton *et al.* (2013) identified some of the main threats of nutrient pollution on terrestrial ecosystems, including ecosystem biodiversity and soil quality. Too much N and phosphorus (P) is known to cause loss of species adapted to nutrient limitations, while too little supply increases the risk of land-use change associated with higher agricultural demands (Sutton *et al.*, 2013). Increased atmospheric N deposition can acidify natural and agricultural soils, while the inability to match crop harvests with sufficient nutrient, in return, leads to depletion of existing nutrients and organic matter (OM). This can further cause land degradation and increased risk of erosion (Sutton *et al.*, 2013).

Due to the fertilizer effects of N in stimulating plant growth, N_r deposition may be acting to influence the atmosphere indirectly by altering the global carbon (C) cycle (Vitousek *et al.*, 1997). However, the current understanding of the impact of increased N deposition on various ecosystems requires further research,

including the C–N interactions and the possible impact of climate change on ecosystems and C sequestration (Shibata *et al.*, 2014, Thornton *et al.*, 2009).

Human interference in the global N cycle for the purpose of food security and energy has increased dramatically the rates of N deposition and thus the levels of additional N available to the biosphere. Due to the changes in rate of N deposition, the usually N-limited terrestrial ecosystems are prone to major transformations. Limited supplies of biologically available N is normal in most natural ecosystems, and many native plant species are adapted to function best under this constraints. One of the most important alterations observed due to atmospheric N deposition is an increasing global threat to biodiversity and ecosystem function (Phoenix *et al.*, 2012); because N supply often limits primary production and other ecosystem processes (Galloway and Cowling, 2002, Vitousek *et al.*, 2002b). New and constant supplies of N dispersed over terrestrial ecosystems could cause a dramatic shift in the dominant species and also a reduction in overall species diversity. This is the result of thriving nitrophilous species, adapted to take full advantage of high N conditions, which will out-compete and ultimately exclude those plants adapted to low N availability (Bobbink *et al.*, 1998, Dise and Stevens, 2005, Phoenix *et al.*, 2006). Secondary factors associated with enhanced N supply can manifest as soil acidification and plants susceptibility to herbivores, frost damage and drought (Phoenix *et al.*, 2006). At the same time, abundant N could lead to new scarcities and potential for release of other elements in soil that have the potential for increased toxicity, further promoting the species best adapted to these novel conditions (Cleland and Harpole, 2010).

Thus, as a result of increased deposition of air-borne N pollutants, many changes will occur in plant growth, interspecific relationships and soil-based processes which are strongly regulated by complex biological and microbiological processes in the N and C cycle (Bobbink and Hettelingh, 2010). Given that soils represent a major store of C, N, P and micronutrients, their sound management is essential to address global food security challenges and minimize nutrient losses to the environment that can pollute air and water and

most importantly potential loss of C from the soil storage, with feedback to the climate. Threats to soil quality including soil compaction, erosion, acidification, salinization, contamination, and OM decline, could impact soil C, N and P losses to water and air (Sutton *et al.*, 2013).

All these further potential harmful effects of excess N availability to the terrestrial ecosystems have been investigated by field and laboratory experiments, with a view to assess the responses of different ecosystems to these changes. The results can then be used to make recommendations regarding the direct management required to offset some of these impacts. Much of the present research is orientated towards investigating the impacts of excess N availability on biodiversity and ecosystem services, and less on the soil C storage in the affected ecosystems. However, even less is known on the effects of the interaction between these factors and what their implications are for climate change mitigation. The current project aims to bridge some of these gaps and bring further understanding to some of the processes governing the observed impacts on terrestrial ecosystems, with potential for feedback to global climate.

1.1.3. Summary

Terrestrial ecosystems are changing and this leads to implications for the organisms present and the processes taking place within them. It is thus important to understand what these changes mean for ecosystem services, including C and nutrient cycling. Knowledge has progressed from the early 1980s when the concept of global warming was first identified and the human influence was acknowledged as one of its prime drivers. Understanding the changes in nutrient dynamics and C cycling in soils are fundamental to accurately predict the future climate change and ecosystem response to N additions.

1.2. CARBON AND NUTRIENT CYCLING IN TERRESTRIAL ECOSYSTEMS

1.2.1. Key carbon cycle processes

Earth's biosphere is continuously changing which is reflected onto its biogeochemical cycles. One of the most important of these cycles and under investigation in this project is the terrestrial C cycle. The two main processes driving the terrestrial C cycle are photosynthesis and respiration (Schlesinger, 1997). Photosynthesis represents the process by which C enters the biosphere, vital for the growth and development of plants, through the production of carbohydrates from CO₂ and water in the presence of chlorophyll and light energy (Raven *et al.*, 1999). On the other hand, respiration is the reverse process by which C is returned to the biosphere in cellular respiration, through complete breakdown of sugar or other organic compounds to CO₂ and water (Raven *et al.*, 1999).

Some of the vital ecosystem C exchange processes are illustrated in Figure 1.2 and can briefly be summarised as: C inputs through photosynthesis, and its release to the atmosphere via multiple respiratory fluxes. The C inputs to the terrestrial system, through the process of photosynthesis, is then allocated to different parts of the plant, followed by respiration in leaves, stems and roots which releases the C back to the atmosphere. About half of the C taken up by photosynthesis may be released through plant respiration. Another vital C flux is represented by the large amount of OM entering soils each year as leaf and root litter and suffering multiple transformation within the soil system.

The main research question under investigation in this project is the large amount of C stored in soils as OM and its potential release back to the atmosphere, due to an enhanced decomposability attributed mainly to higher temperatures (Street *et al.*, 2007). Above-ground, CO₂ is released from leaf and stem respiration, whereas belowground, CO₂ is released through root

respiration, as well as microbial respiration associated with the decomposition of SOM (Kuzyakov, 2006). The uncertainty rests in the fact that root respiration and the respiration of soil microbes may respond very differently to climate change. Therefore, trying to measure how much CO₂ is being released from the soil surface and each of its components can become problematic (Hartley *et al.*, 2007).

The terrestrial C cycle goes through a series of minicycles, causing the atmospheric CO₂ concentration to decrease during the day and summer, or to increase at night time and during winter (Schlesinger, 1997). The reduction in atmospheric CO₂ concentrations are explained by photosynthesis exceeding respiration by decomposers, while the opposite is true at night and in the winter when photosynthesis stops due to the absence of light or the fact that plants are dormant. The following sections will synthesise current understanding of the key processes groups, essential for clarifying the implication of climate change on terrestrial C storage, investigated in this project.

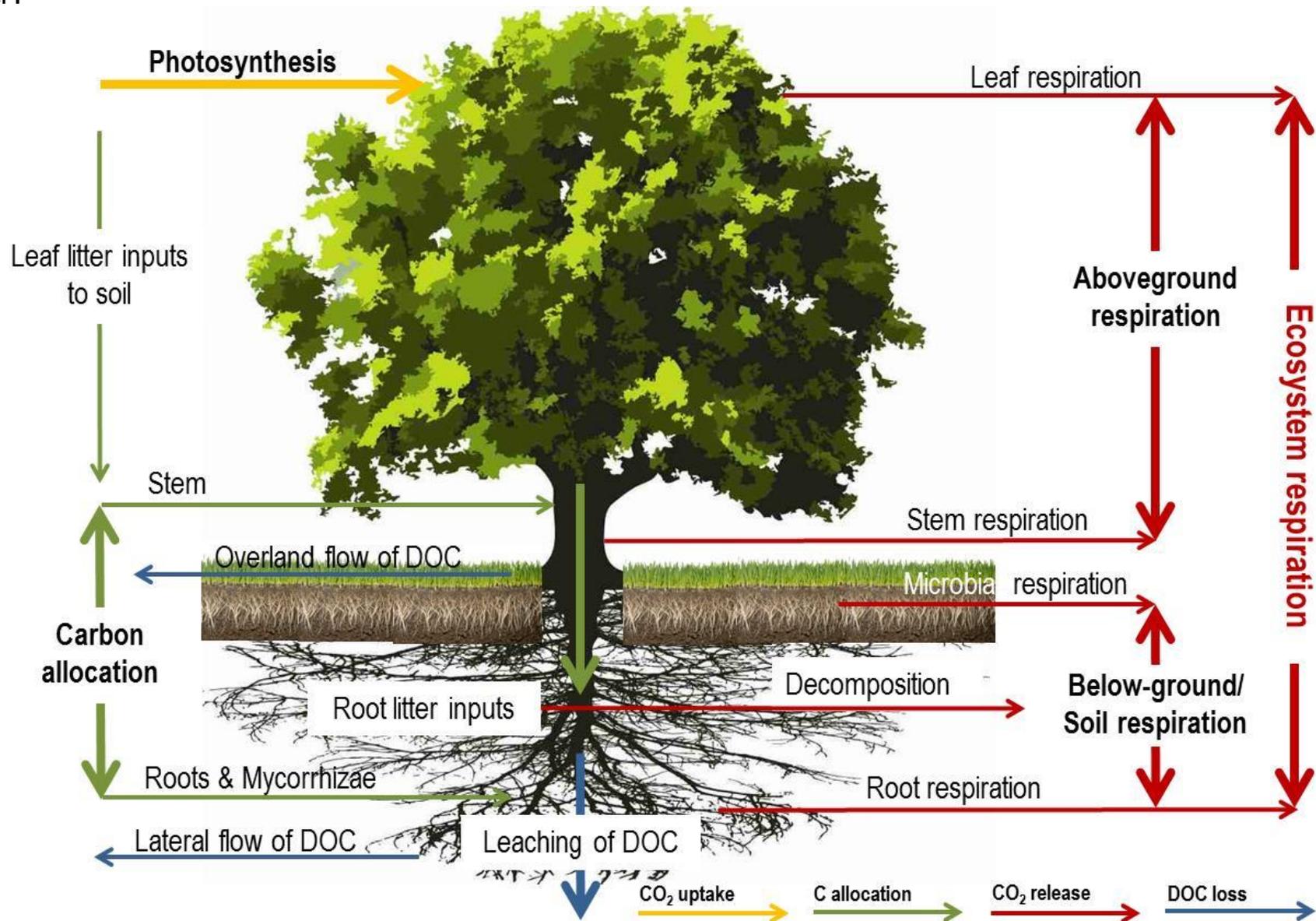


Figure 1.2. Diagram of key ecosystem C exchange processes. C inputs are indicated by the orange arrow, C movement and allocation through the ecosystem are represented by the green arrows, C release is indicated by the red arrows, and dissolved organic C (DOC) loss by leaching or flow is shown in blue arrows.

1.2.1.1. *Photosynthesis and net primary productivity*

Terrestrial plants can fix CO₂ as organic compounds through photosynthesis, a C flux also known at the ecosystem level as gross primary production (GPP) (Beer *et al.*, 2010, Chapin III *et al.*, 2011). The C fixed directly through photosynthesis supports plant growth and produces OM that is consumed by animals and soil microbes. The C derived from photosynthesis makes up about half of the OM on Earth (Chapin III *et al.*, 2011). The C concentration in OM is also variable within plants, but averages about 45 % of dry weight in herbaceous tissues and 50 % in wood (Gower *et al.*, 1999).

The complex chemical processes taking place during the photosynthesis can be summarised by *Eq.1*, as:



Terrestrial GPP is the largest global C flux, and it drives several ecosystem functions, such as respiration and growth (Beer *et al.*, 2010). Net ecosystem exchange (NEE) of CO₂ accounts for the imbalance between GPP and ecosystem respiration (ER), and is defined as either a positive or negative net flux of CO₂ to the atmosphere. For the purpose of this project the atmospheric sign convention was adopted and the NEE of CO₂ is represented by *Eq.2*, as:

$$[Eq.2] \quad \text{Net Ecosystem Exchange (NEE)} = \text{Ecosystem respiration (ER)} - \text{Gross Primary Productivity (GPP)}$$

As a consequence, a positive NEE signifies a net emission of CO₂ from the plant–soil ecosystem to the atmosphere, whereas a negative flux indicates the uptake by the same system (Elsgaard *et al.*, 2012).

Eq.3 represents the relationship between C uptake and C release, quantifying the net primary production of terrestrial ecosystems:

$$\text{[Eq.3] Net Primary Productivity (NPP) = Gross Primary Productivity (GPP) - Plant Respiration (R}_P\text{)}$$

The accumulation of OM as biomass per unit of land represents a measure of NPP, expressed in $\text{g m}^{-2} \text{y}^{-1}$. The C content accumulated in proportion of 45 % – 50 % by C fixation can be calculated by halving the OM accumulation in plant tissue (Schlesinger, 1997). NPP refers to above-ground and below-ground biomass, but the latter is sometimes excluded from studies because of its difficulty to calculate. Studying the NPP is of vital importance, due to the necessity of determining how plant biomass can be altered in response to global climate change (Meehl, 2007).

Once fixed via photosynthesis, C is allocated to the parts of the plant above and below ground, for development and growth. The amount of C allocated to each compartment depends on the stage of the plant development rather than on the plant species (Kuzyakov and Domanski, 2000). Approximately half of the C allocated below-ground is incorporated into root tissue, whereas a third is respired by roots and rhizosphere micro-organisms, while the rest remains in soil and micro-organisms (Kuzyakov and Domanski, 2000). The amount of C allocated and finally stored in soils is of great importance to understanding the extent of climate change. It is known that soils store at the moment at least three times more C as soil organic matter (SOM), than it is found in both plant biomass and the atmosphere (IPCC, 2013). Thus, by understanding the sources and controls of the C released from soils we can fully assess the implications to climate change.

1.2.1.2. *Soil respiration*

Soil respiration represents the largest flux in the terrestrial global C cycle after photosynthesis (Kuzyakov, 2006, Schimel, 1995, Schlesinger and Andrews, 2000). However, there are still considerable uncertainties regarding its actual magnitude, as well as its spatial and interannual variability (Bahn *et al.*, 2010).

Belowground respiration can be separated in two main categories based on the agents of CO₂ production in the soil (Kuzyakov, 2006): (1) respiration by autotrophs - root respiration and (2) respiration by heterotrophs, incorporating rhizomicrobial respiration, microbial respiration of plant residues, and SOM derived CO₂ (Jaoudé *et al.*, 2011). The microbial decomposition of plant litter residues releases CO₂ and nutrients and leads to the formation of SOM. At the global level, the cumulative belowground respiration from the two sources has been estimated to be 55 Gt C y⁻¹, as reported by Prentice (2001) in the 3rd IPCC report. The autotrophic respiration in soils includes the respiration of live roots, their mycorrhizal fungal symbionts and other rhizosphere associated microorganisms dependent on fresh inputs from photosynthesis (Högberg and Read, 2006). Heterotrophic respiration represents the C released from the decomposition of more complex compounds, with a longer residence time in soils, like litter and SOM (Högberg *et al.*, 2009). Changes in soil respiration from either of these components are important due to their influence on C allocation and ecosystem C balance which in turn can affect strongly the terrestrial climate feedback under future conditions (Bond-Lamberty and Thomson, 2010a).

Given that there are obvious dissimilarities between the respiration of soils measured in the field and those measured in the lab, for the purpose of this report and throughout the rest of the thesis, the term soil respiration will represent the heterotrophic respiration linked to decomposition, and belowground respiration will refer to the CO₂ release from the soil surface.

1.2.1.3. Summary

On land, vegetation absorbs CO₂ by photosynthesis and converts it into OM. A fraction of this C is immediately returned to the atmosphere as CO₂ by plant respiration, while the remainder is used for growth. Dead plant material is incorporated into soils, eventually to be decomposed by microorganisms and then respired back into the atmosphere as CO₂. Finally, it is important to mention that C in vegetation and soils can be converted back into CO₂ by fires and the respiration of other organisms within terrestrial ecosystems, whereas

some organic C can be leached or washed into streams, rivers or groundwater as dissolved organic C (DOC). The balance between these processes determines the net exchange of C with atmosphere and is therefore very important regarding the potential for C sequestration. Several key processes remain incompletely understood, like the interaction between nutrients and C cycling in controlling the terrestrial ecosystems capacity to respond to climate change.

1.2.2. Nitrogen and phosphorus cycling

N and P are two of the primary nutrients critical to the development, growth and survival of all living organisms. Their limiting influence in terrestrial ecosystem can have a critical impact on biogeochemical processes. Thus, understanding their key cycling mechanisms is vital for understanding feedback effects driving climate change.

1.2.2.1. *Key nitrogen cycle processes*

Nitrogen (N) is an essential component of proteins, genetic material, chlorophyll, and other key organic molecules. All organisms require N in order to live, being the most common chemical element in living tissues, after carbon (C). Before human activities began to alter the natural cycle, nitrogen was only sparsely available in the biological world. As a result, nitrogen served as one of the major limiting factors that controlled the dynamics, biodiversity, and functioning of many ecosystems (Vitousek *et al.*, 1997). Previous research and climate predictive models failed to incorporate N (Bader *et al.*, 2008). Although over time its importance has been fully recognised and the latest IPCC report (2013) identifies that '*a land nitrogen cycle will reduce the strength of both the concentration–C feedback and the climate–C feedback of land ecosystems*' (Stocker *et al.*, 2013), the extent of the N influence on terrestrial ecosystem response to climate change is still not fully understood (Cosby *et al.*, 2001, Kattge *et al.*, 2009).

N is essential for plant growth and makes up 78 % of the atmosphere. Although N is very abundant in the atmosphere, it is in a form that is inaccessible to most organisms, due to the strength of the triple bond tying the two N atoms together (Galloway *et al.*, 2004). The transformation of N into its many oxidation states is key to the productivity of the biosphere and is highly dependent on the activities of diverse microorganisms, such as bacteria, archaea, and fungi (Butterbach-Bahl *et al.*, 2011). The N cycle processes of *fixation*, *mineralization* and *nitrification* increase plant available N, whereas *denitrification*, *volatilization*, *immobilization*, and *leaching* result in permanent or temporary N loss from the root zone (Figure 1.3). Understanding how N becomes available to organisms and how it changes with each process is important in explaining its effect on other biogeochemical cycles linked to climate change.

a. Biological Nitrogen Fixation

Biological Nitrogen Fixation (BNF) refers to the process of converting the very stable atmospheric N_2 in a biologically available form (Bernhard, 2012), by bonding with hydrogen or oxygen to form inorganic compounds, mainly ammonium (NH_4^+) and nitrate (NO_3^-) (Vitousek *et al.*, 1997). Initially it was thought that lightning and atmospheric deposition were the only processes responsible for N_2 fixation, but later the important role of soil micro-organisms in fixing N_2 necessary for growth was identified (Galloway *et al.*, 2004). Thus, N_2 is biochemically fixed within the soil by specialized micro-organisms like bacteria, actinomycetes, and cyanobacteria.

BNF is catalysed by the enzyme nitrogenase produced by a certain "nitrogen-fixing" bacteria and is the main natural source of fixed N biologically available within the N cycle (Oldroyd and Dixon, 2014). The fixation process requires energy for which N-fixing bacteria often associate with plants to obtain energy-rich organic C compounds in return for the N supply. Symbiotic N_2 fixation is most common in legumes (Lambers *et al.*, 2009), where BNF is dependent on the establishment of symbiotic relationships with an effective *Rhizobium* strain (Graham and Vance, 2003, Ledgard and Steele, 1992). The N-fixing symbiosis

with legumes is important due to its capacity to potentially lower the emissions of two key greenhouse gases: CO₂ and N₂O (Jensen *et al.*, 2012).

b. Decomposition and mineralisation

Mineralisation or ammonification is the microbially-mediated process by which organic N is decomposed to ammonium from OM and plant residues. Following the internal cycling via the plants, N enters the SOM pool by litter production and decomposition (Butterbach-Bahl *et al.*, 2011). The SOM pool can contain N that is not biologically available for uptake, until it is decomposed by soil microbes and transformed in a bio-available form. The decomposition and mineralization of organic N into inorganic forms provide the soil microbes with a C source for respiration and growth.

c. Nitrification, denitrification and leaching

Nitrification is the process by which ammonium (NH₄⁺) or ammonia (NH₃) is oxidized to nitrite (NO₂⁻) and subsequently to nitrate (NO₃⁻) by a restricted group of nitrifying bacteria (Chapin III *et al.*, 2011). These are divided into: autotrophic nitrifiers, that use the energy yielded by the NH₄⁺ oxidation to fix C that supports their growth and maintenance, and heterotrophic nitrifiers, which use the energy derived from the breakdown of OM (Chapin III *et al.*, 2011). Nitrate is the most bio-available form of N, but also highly prone to losses by leaching or denitrification. Denitrification represents a further reduction of both nitrite and nitrate to gaseous nitric oxide (NO) and nitrous oxide (N₂O), and N₂ (Butterbach-Bahl *et al.*, 2011). This process too is initiated by microbes, some fungi and archaea. Nitrogen can be lost from terrestrial ecosystems by leaching as DON from all ecosystems and as nitrate from nitrate-rich ecosystems (Chapin III *et al.*, 2011). The mobility of N in soils largely depends on the form of dissolved N (NH₄⁺, NO₃⁻ or DON). The rate of leaching depends on soil drainage, rainfall, amount of nitrate present in the soil, and plant uptake.

d. Immobilisation

Immobilisation is the reverse of mineralisation. All living things require N; therefore microorganisms in the soil compete with plants for N. Immobilization

refers to the process by which nitrate and ammonium are taken up by soil organisms and therefore become unavailable to plants. Incorporation of materials with a high C to N ratio (e.g. sawdust, straw, etc.), will increase biological activity and cause a greater demand for N, and thus result in N immobilization. Immobilisation can only temporarily lock up N. When the microorganisms die, the organic N contained in their cells is converted by *mineralization* and *nitrification* to plant available nitrate. On the other hand, the plant assimilation of N represents immobilisation for microbes, until the biomass is returned to the soil and N made available again to them by *mineralisation*.

e. Plant uptake

The N available for plant uptake is either nitrate or ammonium (as inorganic nitrogen sources) and amino acids (as organic sources). Conventionally, proteins are broken down into amino acids and then into ammonium (Masclaux-Daubresse *et al.*, 2010). However, many plants can take up amino acids and therefore not depend on full mineralisation. Nitrate reduction takes place in both roots and shoots where the reaction is catalysed by the enzyme nitrate reductase (NR). N uptake by the roots and further N assimilation are integrated in the plant to match the nutrient demand of the whole organism. This rate of the absorption process is controlled largely by the physiologic condition and age of the plant species (Galitz, 2009).

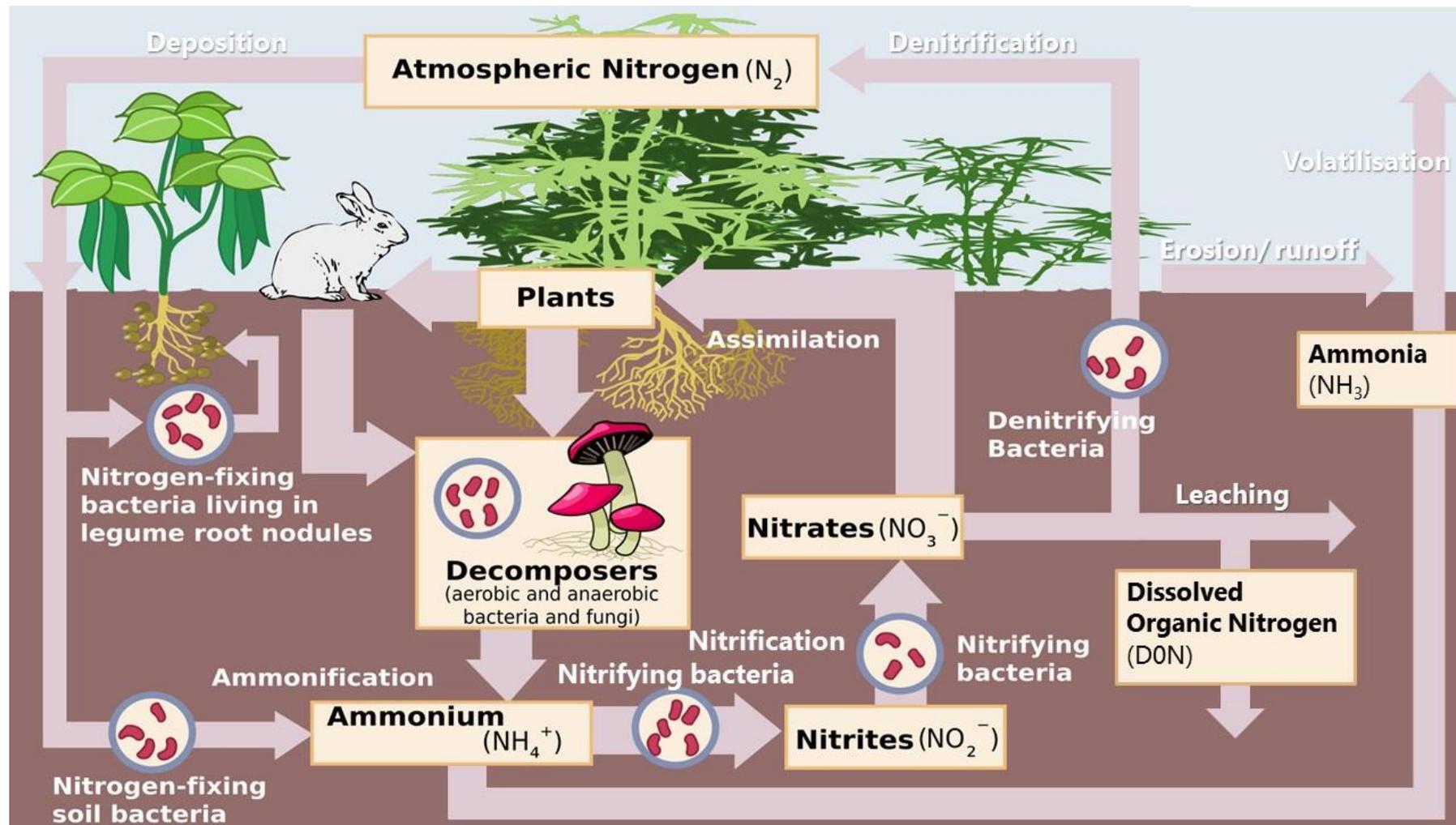


Figure 1.3. Schematic representation of the terrestrial nitrogen cycle. Nitrogen in the environment can be present as organic nitrogen, ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), nitric oxide (NO) or inorganic nitrogen gas (N_2). Nitrogen becomes accessible to plants for growth through processes of fixation, mineralization and nitrification. The opposite reactions of denitrification, volatilization, immobilization, and leaching result in permanent or temporary N losses from the root zone (modified from USA Environmental Protection Agency website).

1.2.2.2. Key phosphorus cycle processes

In contrast to N, the P inputs to natural systems are largely physical and chemical (Figure 1.4). The primary source of P in terrestrial ecosystems is the weathering of parent material during soil development, when P is released mainly from apatite, strengite and variscite minerals (Shen *et al.*, 2011). Physical weathering and erosion of geological material results in a form of P that is typically initially unavailable to biota. Only after this fine material is deposited in continental environments, such as floodplains and delta systems, where it undergoes subsequent chemical weathering and soil development, does it become bioavailable. Thus, the total amount of P weathered from continents may be very different from the amount of potentially bioavailable P (Filippelli, 2002). P created as a result of weathering is estimated globally at 3 Mg, but can vary locally from 0.05 to 5 kg P ha⁻¹ y⁻¹ (Vitousek *et al.*, 2010). Although there are no significant phosphorus-containing gases moving through the atmosphere, additional aeolian inputs of P can be deposited as dust, ash, pollen or sea spray (Campbell and Reece, 2011), but no global estimates can yet be made. However, existing funnel traps methods, were used to calculate aeolian P inputs to the ecosystem and were found in a range 0.07 to 1.7 kg P ha⁻¹ y⁻¹ (Cleland and Harpole, 2010).

The most biologically important inorganic form P is phosphate (PO₄³⁻), which is absorbed by the plant as either H₂PO₄⁻ or HPO₄²⁻ (Shen *et al.*, 2011), and used in the synthesis of organic compounds. Inorganic P accounts for 35 % to 70 % of total P existent in soil (Harrison, 1987) and because soil particles bind PO₄³⁻, the recycling of P tends to be quite localized in ecosystems. Plants can access the bioavailable inorganic P from SOM with the help of symbiotic fungi mycorrhizae and once taken up it is incorporated into plant tissue where it is converted into an organic form (Filippelli, 2008). Organic P represents 30 % to 63 % of the total P present in soil (Harrison, 1987). After the plants die and the tissues decay, organically bound P suffers the same fate as organic litter. Decomposition of biomass happens due to mineralization processes mediated

by soil organisms and plant roots slowly oxidize the OM releasing P as phosphate to soil solutions (Bucher, 2007).

The association between P and SOM makes the understanding of the P cycle a vital component in assessing the limitations on other biogeochemical processes. For example, on centennial time scales, P limitation of terrestrial C uptake could become more severe than the nitrogen limitation because of limited P sources. Root exudates and mycorrhizae can increase the rate at which P is made available to plants, but this biological influence is small compared to the primary importance of physical weathering. Model simulations have shown that after 2100, at high latitudes, a shift from N to P limitation will be possible (Goll *et al.*, 2012). However, in order to fully understand the shift extent and implications for the C and nutrients cycling, further research into their interaction is necessary.

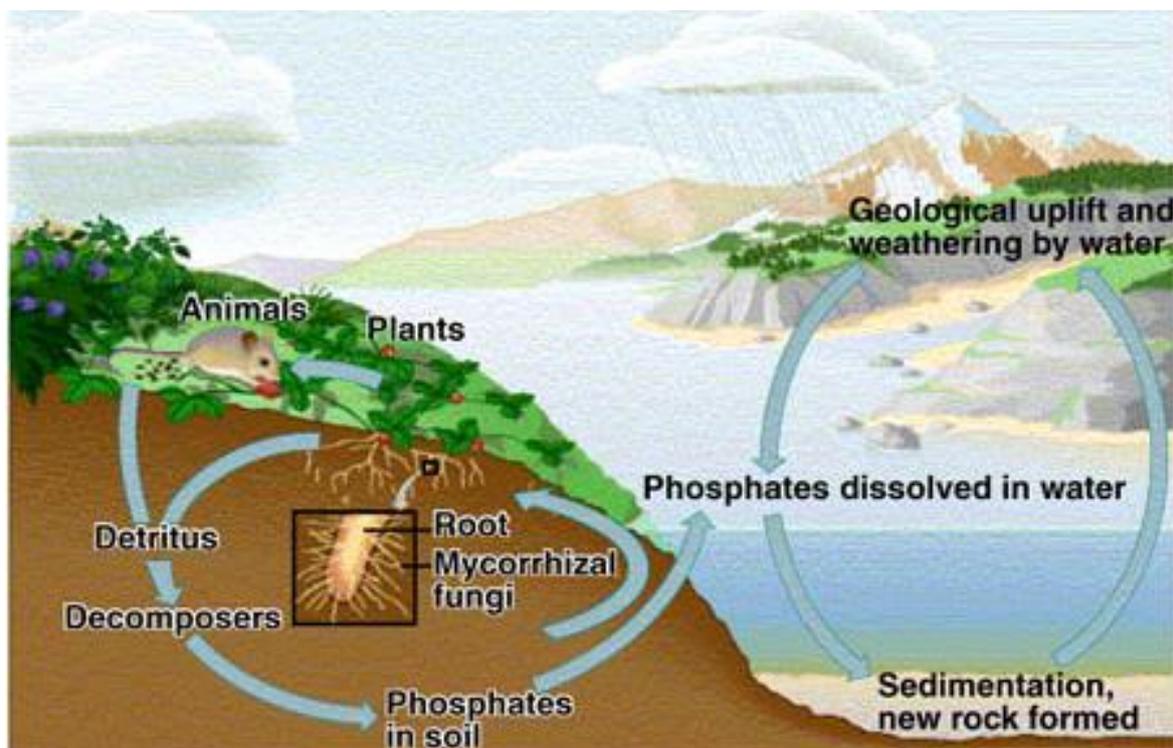


Figure 1.4. Schematic representation of the phosphorus cycle, illustrating the succession of processes in the biosphere. There is no gaseous phase in the phosphorus cycle. Phosphorus is released by the weathering of rocks and minerals or from phosphorus-rich deposits formed in the ocean and migrated to the land as sediments. Then it is taken up by plants and transformed into organic compounds. After plants decay the phosphorus is returned to the soil, where a large part is transformed into insoluble compounds. However, a small part can be lost by runoff to the ocean, as orthophosphates or dissolved phosphorus (*USA Environmental Protection Agency website*).

1.2.3. Interactions between C and nutrient cycling

Enhanced GHGs emissions and land use changes are among the most significant drivers of climate change. By shifting the terrestrial ecosystems from sinks to sources of C there is potential for a positive feedback, thus further accelerating climate change. Due to the significance and vulnerability of the high quantities of C stored in terrestrial ecosystems, it is important to understand the processes controlling this stock. The C and nutrient cycles are both responsible for the sequestration of C as SOM and its release back to the atmosphere as CO₂. Moreover, an efficient interaction between these cycles could be the key to sustained C uptake by terrestrial ecosystem and thus, climate change mitigation. For this reason these cycles cannot be studied in isolation, as explaining the full implications of climate change resides in understanding the linkages between their processes. The conceptual model illustrated in Figure 1.5, summarises the interactions between the C and N cycles that are central to the experimental design of this project. Elucidating these processes is essential for accurate prediction of future climate change.

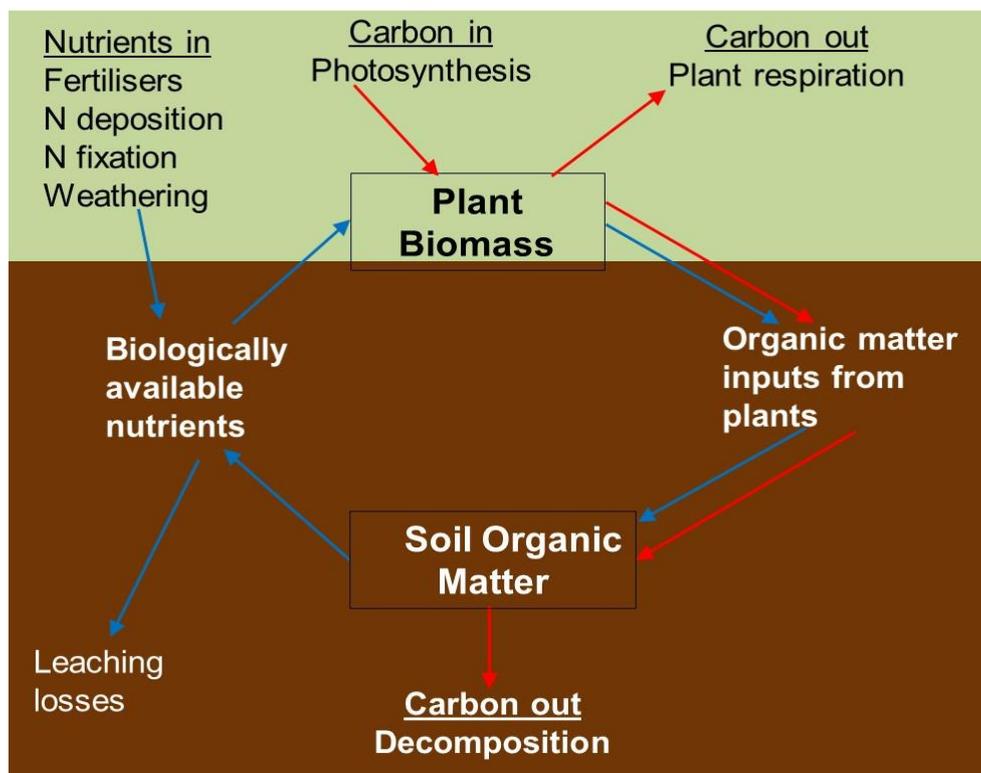


Figure 1.5. Conceptual model representing the C and nutrient cycle interactions, illustrating the interdependence of their key processes and the potential for ecosystem C sequestration

1.2.3.1. *Links between decomposition and plant growth*

Mitigating climate change by sequestering more C in the terrestrial ecosystems has long been the aim of numerous studies and experimental work (Lal, 2008, Powlson *et al.*, 2011b, Waldrop *et al.*, 2004, Wilmking *et al.*, 2006). Either stored in soil as SOM or as biomass, the C cycling through the system is subject to a variety of transformations driven by the balance between decomposition and plant growth. The cycles of N and C are tightly coupled with each other owing to the metabolic needs of organisms for these two elements. Changes in the availability of one element will influence not only biological productivity but also availability and requirements for the other element (Gruber and Galloway, 2008) and, in the longer term, the structure and functioning of the ecosystem as well.

Decomposition of SOM is the most important process supplying N and P to plants, and decomposition releases ten times as much C into the atmosphere as fossil fuel combustion. Moreover, multiple species typically contribute to pools of plant litter, and researchers have reported strong positive and negative effects of diverse plant litter mixtures on litter mass loss, soil respiration, and soil N dynamics. It is therefore critical to develop a better mechanistic understanding of how plant litter diversity influences decomposition, and specifically, how plant diversity affects soil C and N dynamics (Meier and Bowman, 2008).

Based on new experimental results and modelling, there is growing evidence that nutrient shortage will limit the effect of rising atmospheric CO₂ on future land C sinks (Stocker *et al.*, 2013). Soils with large pools of organic C and high C:N ratios are generally associated with N accumulation and tend to export less nutrients than soils with low C:N ratios (Aber, 1992). When N_r availability is elevated in an N-limited system (e.g., through atmospheric N deposition), soil inorganic N is readily utilised by plants, resulting in increased C uptake (Gruber and Galloway, 2008) and reduced below-ground allocation of C (Deegan *et al.*, 2012). Elevated N_r in soil can also change the soil microbial community by, for

example, a reduction of fungal:bacterial biomass ratios (Högberg *et al.*, 2004). These studies emphasize the importance of taking N–C interactions into account when considering the possible impact of climate change on ecosystems, C sequestration, and in the development of earth system models (Thornton *et al.*, 2009).

Crucially, the effect of N limitation on vegetation growth and C storage under elevated CO₂ conditions is the strongest effect of the natural and disturbed nitrogen cycle on terrestrial C dynamics (Bonan and Levis, 2010, Zaehle *et al.*, 2010). Nevertheless, there are limits to how much plants can grow due to increased N availability, because even when the natural N deficiencies in an ecosystem are relieved, plant growth can become limited by the reduction of other resources including P, calcium, or water (Vitousek *et al.*, 1997). The ecosystem saturation with one nutrient can create a deficit in all the other supplies to soil, plants, and microbes leading to disturbed biogeochemical cycles and potentially loss of excess nutrients to streams, groundwater, and the atmosphere (Butterbach-Bahl *et al.*, 2011, Vitousek *et al.*, 1997).

In addition, published studies suggested that in acid soils, competition between plants and nitrifying microbes is the driving factor behind nitrification that happens mainly when there is an excess of ammonium relative to the plant demand (Aber *et al.*, 1998, Averill *et al.*, 2014, Hodge *et al.*, 2000, Kaye and Hart, 1997). Microbes can also decrease the soil C:N in the absence of fresh inputs, by rapidly mineralising SOM and encouraging C to leave the system at a faster rate than N. This change in stoichiometry could then affect fungal abundance because of a smaller demand of N per unit biomass C accumulation than bacteria (McFarland *et al.*, 2013).

1.2.3.2. *Mycorrhizal fungi*

A very important link in the soil-plant interaction system is represented by mycorrhizal symbiosis. Most plants have roots colonised by mycorrhizae (Campbell and Reece, 2011), which are mutualistic associations between the roots and fungi. Mycorrhizal fungi are the organisms responsible for helping plants access nutrients, especially N and P. Harley (1971), was among the first to prove that mycorrhizal fungi are highly dependent on the C supplies from recent photosynthesis rather than carbonaceous detritus from the litter fall or dead roots. Later, insight into the intricate links of belowground processes advanced to show substantial increase in fungal growth for young trees receiving high CO₂ concentrations (Meir *et al.*, 2006).

Mycorrhizal fungi receive growing attention, as they could also demonstrate a critical role in C sequestration of soils, as an important part of the global C cycle, (Averill *et al.*, 2014). As the predominant form associated with terrestrial plants (Smith and Read, 2010), the arbuscular mycorrhizal (AM) fungi are able to directly influence soil C dynamics through the growth and turnover of extra-radical hyphae within bulk soil (Zhu and Miller, 2003). This component of the AM, might prove essential in studying the extent of climate change impact on terrestrial ecosystems, due to the potential negative feedback to rising atmospheric CO₂ and increasing temperatures, due to a stimulation of extra-radical mycorrhizal hyphal density under a warmer climate (Staddon *et al.*, 2002).

The resilience and good functioning of the symbiosis depend on numerous factors and the interaction between changing environmental circumstances, such as physicochemical properties of the soils that are not yet fully understood. This gives the opportunity to address unanswered issues that need addressing, such as the driving interaction processes between the nutrient accessibility, facilitated by the mycorrhizae, and the ecosystem's capability to store C.

1.2.4. Summary

Despite recent progress in understanding C and nutrient cycling in terrestrial ecosystems, there remain significant gaps in understanding that limit the potential to predict anthropogenic impacts on terrestrial ecosystems. There remains uncertainty regarding the fate and dynamics of the large global terrestrial C store (Davidson and Janssens, 2006, Kirschbaum, 1995) and the response of C sequestration in the terrestrial ecosystems to global warming (Heinemeyer *et al.*, 2007). It is not clear whether we can continue to rely on the major C sink represented by the terrestrial ecosystems, as some of the fundamental processes sustaining this uptake might diminish (Schimel *et al.*, 2001). These uncertainty are especially important because, as an essential regulator of atmospheric chemistry and climate, the terrestrial biosphere has the potential to demonstrate in just a few decades important transformations of vegetation cover and interactions between its systems (Arneeth *et al.*, 2010). That is why it is vital to understand the magnitude of the terrestrial C sink and potential contributions from its processes (Schimel *et al.*, 2001), along with the impacts on the interactions between terrestrial ecosystems and atmosphere (Arneeth *et al.*, 2010).

Furthermore, it is still unknown how these beneficial interactions between C and nutrient cycles could be affected by the shifting ecosystem functionality due to global change processes.

1.3. NUTRIENT AND CARBON CYCLING IN THE CONTEXT OF GLOBAL CHANGE

1.3.1. Elevated CO₂

A key question is how will interactions between nutrients cycles respond to the atmospheric enrichment of CO₂ and shifting nutrient availability, expected over next century. On physiological grounds, almost all models predict stimulation of C assimilation and sequestration in response to rising CO₂, called 'CO₂ fertilization' (DeLucia *et al.*, 2005, Oren *et al.*, 2001). Free Air CO₂ Enrichment (FACE) and chamber studies have been used to examine the response of ecosystems to large (usually about 50 %) step increases in CO₂ concentration. On average, net CO₂ uptake has been stimulated, but not as much as predicted by some models. Other factors like nutrients can limit plant growth and reduce response to CO₂. There is a large range of responses, with woody plants consistently showing NPP increases of 23 % to 25 % (Norby *et al.*, 2005), but much smaller increases for grain crops (Ainsworth and Long, 2005). Overall, about two-thirds of the experiments show positive response to increased CO₂ (Ainsworth and Long, 2005), though it is not yet clear how strong the CO₂ fertilization effect actually is.

The increased C storage in terrestrial ecosystems not adversely affected by land use change is predominantly caused by enhanced photosynthesis stimulated by the fertilization effect of atmospheric CO₂ enrichment (Le Quere, 2010, Stocker *et al.*, 2013). However, nutrient limitation is hypothesized as a primary cause for reduced or lack of a CO₂ fertilisation effect observed on NPP in some experiments (Dukes *et al.*, 2005, Luo *et al.*, 2004, Norby *et al.*, 2010). N and P are very likely to play the most important role in this limitation of the CO₂ fertilisation effect on NPP, with N limitation prevalent in temperate and boreal ecosystems, and P limitation in the tropics (Goll *et al.*, 2012, Luo *et al.*, 2004, Vitousek *et al.*, 2010). Micronutrients interact in diverse ways with other nutrients in constraining NPP such as molybdenum and P in the tropics.

Understanding the links between C and nutrient cycling is therefore critical for predicting ecosystems response to elevated atmospheric CO₂ concentrations.

Coupled carbon-cycle climate models indicate that less C is taken up by land as the climate warms, constituting a positive climate feedback. Higher temperatures may result in faster decomposition of soil C (IPCC, 2013), but while this may release more CO₂, the increased nutrient mineralisation may promote plant growth. Therefore, again, carbon-nutrient cycle linkages are central to understanding the responses of terrestrial ecosystems to climate change. Thus, it is understood with high confidence that the CO₂ fertilisation effect will lead to enhanced NPP, but significant uncertainties remain on the magnitude of this effect, given the lack of experiments outside temperate climates (IPCC, 2013).

1.3.2. Climate Change

Climate change knowledge is based around the information coming from the long record of atmospheric CO₂ concentrations (IPCC, 2013) and their influence on mean global temperatures (Solomon *et al.*, 2007), and fundamental understanding of the physics of climate. Great importance is placed on the future impact that increased atmospheric temperatures will have on the efficiency with which terrestrial ecosystems help reduce the present atmospheric CO₂ levels (Le Quere, 2010). On these elements, and especially on the interactions between them, rests the key to estimating the terrestrial C cycle behaviour to this century warming predictions, with potential to either mitigate or intensify current atmospheric increases of CO₂ levels, through climate change feedbacks (Jaoudé *et al.*, 2011).

The IPCC considers increased decomposition of SOM due to soil warming as an important potential feedback to climate change (IPCC, 2013). However, detecting changes in the SOM stock of terrestrial ecosystems under global change can be difficult, because SOM has a complex and heterogeneous composition due to its association with various degrees of mineralised soil

elements with different physical and chemical stabilities (Del Galdo *et al.*, 2003). Thus, in order to understand how an increase in temperature stimulates changes in the SOM pools, one needs to understand the composition and behaviour of the SOM under investigation. Currently, C in SOM accounts for 80 % of the terrestrial C pool and is regarded as an important C sink with the potential to help offset the greenhouse effect (Maia *et al.*, 2010).

Another important C pool is the dissolved organic carbon (DOC), which is usually missed from calculations of C budgets in terrestrial ecosystems (Brazier *et al.*, 2014), that can potentially mislead global-scale predictions of C stocks. The loss of C from the system can be a useful indicator of the ecosystems capacity to sequester and store C in the long term. It is also important to be able to quantify the role of this labile organic matter fraction in regards to the C and N transformations in soil (Cook and Allan, 1992). Different studies have found that the loss of C as DOC can be reduced by adding nutrients to the soil (Jones and Donnelly, 2004), although different soil amendments can have similar beneficial effect (Laird *et al.*, 2010). It is thus important to investigate all the factors that could lead to a reduction of C loss from terrestrial ecosystems and increase the soil capacity to store C, under changing climate conditions.

It is expected that even small changes to the SOM stock under global warming can have a massive influence on atmospheric CO₂ concentrations (Jaoudé *et al.*, 2011, Reichstein *et al.*, 2005). Thus, it is essential to expand knowledge on the effects global warming has on SOM decomposition (Cheng *et al.*, 2011). It has been suggested that warming can result in huge increases in SOM decomposition and the nutrients released may stimulate uptake, but the exact magnitude and limitations of these interactions are still unknown, making it vital to explore their potential implications for climate change.

Kirschbaum (1995), investigated the effects climate warming could have on future dynamics of the large soil C store, with emphasis on the need for new knowledge regarding the relative temperature sensitivity of the SOM decomposition rates. The matter has received considerably more interest with

time, because of the important role SOM plays in the C cycle, with potential feedbacks to climate change (Davidson and Janssens, 2006). There are other factors that can influence the susceptibility of SOM to warming, including the chemical and physical protection in soil (Figure 1.6). Six *et al.* (2002) offers a good representation of the processes that transform SOM from a free-unprotected form to a physically protected and finally a recalcitrant-biochemically protected state. Depending on the type of input and quality, time from input, temperature variations and temperature sensitivity, SOM becomes more and more protected with each transformation that it suffers. Thus, moving from the relatively small labile C pool (top box) to the bigger recalcitrant one (middle and bottom box) the SOM becomes less affected by warming and potentially more resistant to decomposition (Six *et al.*, 2002).

Recent studies have significantly advanced understanding of warming effects on nutrient utilisation, primary productivity (Janssens *et al.*, 2010, Magnani *et al.*, 2007, Raich *et al.*, 2006, Van Groenigen *et al.*, 2006) and OM decomposition (Bond-Lamberty and Thomson, 2010b, Hartley *et al.*, 2007, Jones *et al.*, 2006, Maia *et al.*, 2010), as separate processes. Nevertheless, the effect of warming on the interaction between these effects remains a key knowledge gap.

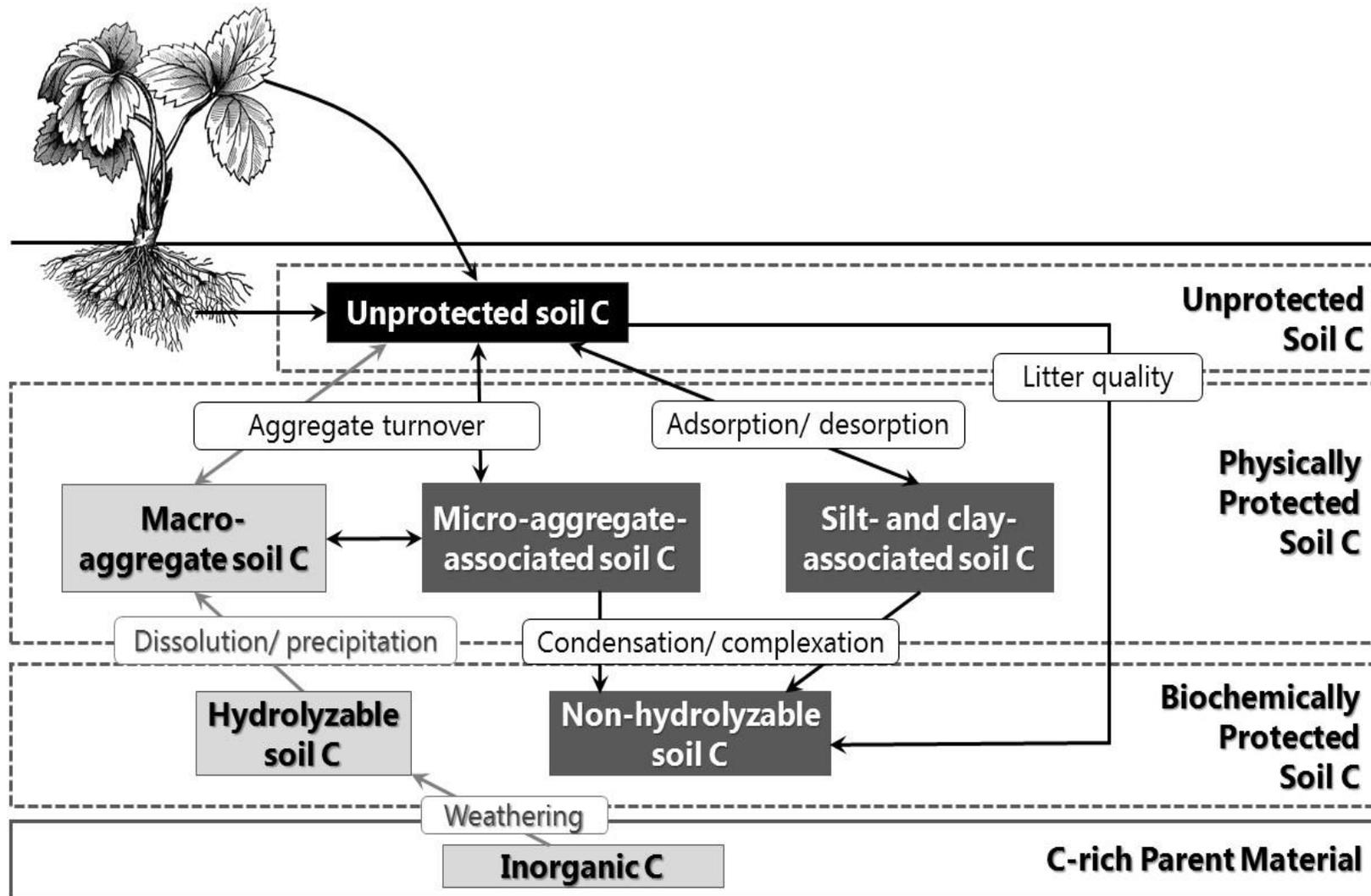


Figure 1.6. A schematic representation of the soil organic matter (SOM) dynamic and soil inorganic C (SIC), with measurable pools, in relation to their quality and availability (modified from Six *et al.* (2000)).

1.3.3. Grassland management for carbon sequestration

Due to increased demand for food and crops, many ecosystems have been transformed from natural to agricultural, improved or semi-natural ecosystems. However, this land use change could affect basic ecosystem functionality, nutrient dynamics and soil properties. In the UK, a considerable number of habitats have been affected by land use change and are now in one form or another of improved or managed ecosystem (Figure 1.7). For these reasons, extensive research on macronutrient cycling in agricultural systems aims to optimise soil and nutrient management for enhanced production and to reduce the impacts on the environment and ecosystem services (Dungait *et al.*, 2012).

Considering the importance of these ecosystems in the terrestrial C dynamic, further emphasis must be added on the fate of the SOM content and capacity to store C, depending on the new practises employed. The main challenge is managing the supply and utilisation of nutrients (N and P) and water to increase the productivity of agricultural systems in a sustainable way, whilst minimising impacts on other ecosystem services, such as clean water and air, biodiversity and C sequestration (Powlson *et al.*, 2011a). For this reason, conversion from either cultivation or native vegetation into grassland could also offers possibilities for mitigation of greenhouse gas emissions by sequestration of atmospheric C (Conant *et al.*, 2001, Dungait *et al.*, 2012).

One of these ecosystems heavily relied upon for food and forage production are grasslands, which according to Figure 1.7 represent a vast majority of the ecosystems in the UK. Grasslands are important for their high SOM content that supplies plant nutrients, increases soil aggregation, limits soil erosion, and also increases cation exchange and water holding capacities (Bellamy *et al.*, 2005, Haygarth *et al.*, 2006). Many management techniques intended to increase forage production may potentially increase SOM, thus sequestering more C. One of these techniques implies sowing mixed forage with legumes/forbs in order to reduce the need for inorganic N additions and its potential long term negative impacts on the ecosystem. Research indicates that in stands with

25 % or more legume cover, no additional N is needed for the plant growth and increased yields.

In addition to increasing forage production, sowing grasses and legumes often results in increased belowground production and it can have implications for SOM dynamics (Crawford *et al.*, 1996), which can lead to increased belowground C inputs and soil storage. At the same time, the introduction of legumes in a grassland can increase soil nitrogen, resulting in superior soil fertility, further increasing aboveground and belowground production (Watson *et al.*, 2002). Research indicates that it is likely sowing mixed forage species increases total plant–soil system C, thus potentially sequestering atmospheric C (Conant *et al.*, 2001). These rates of soil C sequestration can be influenced by many factors, such as management changes and history, but also by climate and native vegetation. Due to their relatively high C sequestration potential and extensive habitat coverage, improved managed grasslands could be a substantial global sink for atmospheric C (Conant *et al.*, 2001).

The remaining uncertainties, regarding the C sequestration potential in improved managed grasslands can be understood by further assessing the factors controlling the fundamental processes involved.

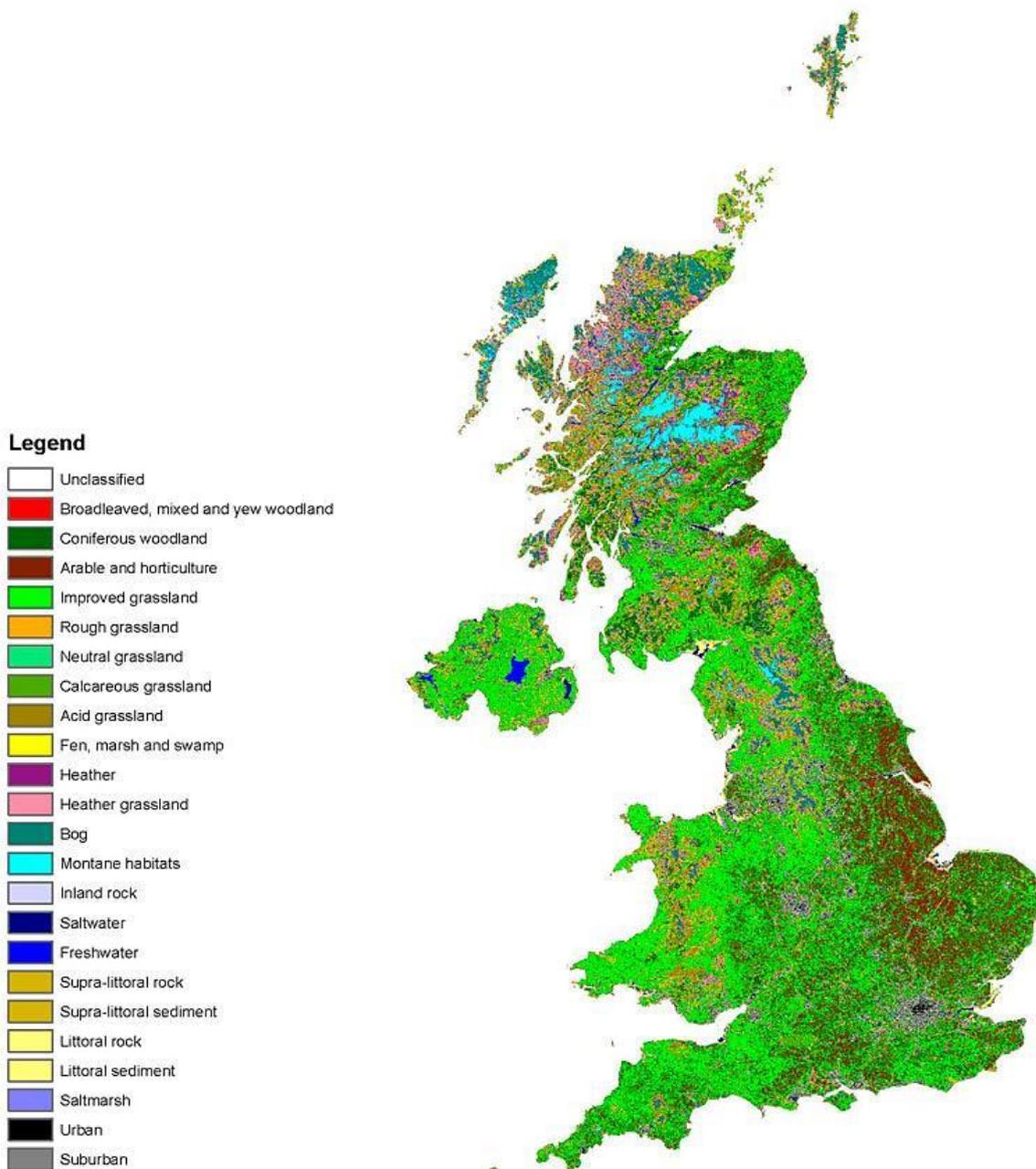


Figure 1.7. UK map illustrating the natural and man-made habitats, emphasizing the extent of the improved grasslands in comparison with those unimproved (*source: NERC website*).

1.3.4. Carbon, nutrients and warming

C is fundamentally linked with other elemental cycles and that is reflected in the ratio of these elements, which can be determined by the shift in nutrient requirements for tissue building and decomposition (Vitousek *et al.*, 2002a). The availability of nitrogen, which is limited in many ecosystems, plays a critical role in controlling NPP (Arneeth *et al.*, 2010). The balance between NPP, soil heterotrophic C decomposition and disturbance depend on the net land-C uptake, through a stimulation of photosynthesis by increasing atmospheric CO₂ concentrations and lengthening growing seasons (Friedlingstein *et al.*, 2006). For this reason it is useful to explain some basic processes involved in the soil-plant interactions in the context of climate change, because it can influence the soil C storage as a result of increasing nutrient-use efficiency under high [CO₂] (Polley *et al.*, 1995) and can potentially cause even further C loss (Melillo *et al.*, 2002).

The basic biochemistry of photosynthesis implies that stimulation of growth will reach saturation under high CO₂ concentrations conditions and growth will be further limited by nutrient availability (Dukes *et al.*, 2005). C storage by terrestrial plants requires net assimilation of nutrients, especially N, a primary limiting nutrient at middle and high latitudes and an important nutrient at lower latitudes (Vitousek *et al.*, 1998). Hungate *et al.* (2003), argue that 'soil C sequestration under elevated CO₂ is constrained both directly by N availability and indirectly by nutrients needed to support N₂ fixation', while Reich *et al.*, 2006b conclude that 'soil N supply is probably an important constraint on global terrestrial responses to elevated CO₂'. This view appears to be consistent with other more recent studies (Norby and Iversen, 2006) and with at least some of the FACE data (Ainsworth and Long, 2005), further complicating estimation of the current effects of rising CO₂ on C sequestration globally.

Anderson (1992) suggested that the rise in CO₂ leading to increases in plant biomass, and hence terrestrial C storage, is an oversimplification, since belowground C storage dominates in some terrestrial ecosystems. In addition,

warming-induced changes in SOM regulate the availability of N for plant growth and ultimately influence the NPP of terrestrial ecosystems (Cheng *et al.*, 2011). Critically, it has been demonstrated in a soil warming experiment in a temperate forest, that C losses from increased rates of decomposition can be compensated for by increased tree growth as a result of greater rates of nutrient mineralisation (Melillo *et al.*, 2002). Furthermore, increased decomposition rates at high temperatures may provide some of the nutrients required to sustain a strong elevated CO₂ response (Zaehle *et al.*, 2010).

In summary, the current increased terrestrial ecosystem uptake potential is related to the CO₂ fertilisation (Le Quere, 2010). However, studies suggest that, in the future, the effectiveness of the terrestrial sink may decline due to nutrient limitation (Le Quere, 2010). The potential conversion of the global terrestrial C sink to a source is critically dependent upon the long-term sensitivity of soil respiration to global warming, and whether soil C losses can be compensated for by plant C gains due to increased nutrient availability. Ultimately, there remains considerable debate and unanswered questions regarding how links between nutrient and C cycling control terrestrial ecosystem responses to global change.

1.3.5. Summary

Arguments made so far demonstrate that nutrient cycling plays a key role in controlling the fate of the soil C, though there is no full understanding of the consequences of these interactions. The experiments designed in this PhD are aimed at determining how links between C and nutrient cycling control C storage in terrestrial ecosystems and the responses to changes in temperature. This has recently been identified as one of the major areas of uncertainty in modelling C-cycle feedbacks to climate change (Arneth *et al.*, 2010). Although no model output will be used in this project, the aim is to produce valuable information and explicit process understanding that could help increase the quality of data input in these models. This will partially be achieved by placing the responses of different processes into the context of the proposed

Conceptual Model developed in this chapter (Figure 1.5), and further discussed in Chapter V.

As part of the experimental design, this project will address the need for understanding soil C cycling, based on the manipulation of nutrients and temperature, and determine the role played by these in the potential increase of terrestrial C stocks. Consequently, the focus of this review will now be shifted towards some of the terrestrial C cycle processes that remain uncertain in their dynamics, in the context of global warming.

1.4. RESEARCH REQUIREMENTS

The preceding review has demonstrated that there is growing recognition that links between nutrient and C cycling need to be considered explicitly when investigating how all global change drivers will affect the function of terrestrial ecosystems. This thesis focuses on developing important understanding of how nutrient availability controls plant growth, decomposition rates in soils and ecosystem C storage, and how these are in turn affected by temperature

1.4.1. Nutrient availability and ecosystem carbon storage

There have been numerous studies dealing with the effects of increased nutrient availability on terrestrial ecosystem biodiversity (Arroniz-Crespo *et al.*, 2008, Phoenix *et al.*, 2003) and productivity (Phoenix *et al.*, 2012). However, much less emphasis has been placed on how nutrient manipulations affect soil C storage, especially in grassland ecosystems (Averill *et al.*, 2014, Stewart *et al.*, 2009). Atmospheric N deposition can be simulated by mineral nutrient additions of N, while P availability can also be manipulated to allow for investigation of how nutrient availability controls net ecosystem C exchange and soil CO₂ and ultimately ecosystem C storage.

1.4.2. Nitrogen fixation and carbon uptake

A change in the amount and source of nutrients can affect the dynamics of the C cycling in the terrestrial ecosystem. Without the use of fertilisers, in natural ecosystems, N availability is increased by the process of N fixation. Changes in the availability of biologically fixed N can also have consequences on the C uptake and storage in terrestrial ecosystems. A review by Cleveland *et al.* (1999) estimates that biological N fixation by legumes in natural systems can fix approximately 195 Tg N y⁻¹, while global N fixation rates in agricultural systems were calculated at around 50 Tg y⁻¹, which is about half the annual application of mineral fertiliser N to agricultural lands (Unkovich *et al.*, 2008). Besides

higher nutrient availability for plants, enhanced biomass production and soil fertility, biologically fixed N availability could boost the capacity to store more C in soil and biomass. It is important to determine if promoting rates of N fixation can increase the capacity of terrestrial ecosystems to fix and sequester C from the atmosphere.

1.4.3. Nutrient availability controls over ecosystem responses to warming

Climate change has the potential to affect all the components of the C-nutrient interaction cycle (Figure 1.5), and to shift the basic processes controlling these components. One of most worrying aspects of climate change is the potential for positive feedbacks to accelerate the temperature increase. Many studies have attempted to explain the extent of warming impacts on a number of key processes in the C and N cycles, but a consensus has not yet been reached.

The work presented in this thesis endeavours to bring further understanding into the functionality of the terrestrial ecosystem as a whole, driven by changes in temperatures and nutrient availability. Ecosystem processes have previously been investigated under the influence of a series of factors like nutrient availability and warming in field conditions and here are further explored in a controlled laboratory environment. By studying the effects of 1) inorganic nutrient additions, 2) altered rates of BNF associated with changes in plant biodiversity, and 3) interactions between nutrient availability and SOM responses to warming, this PhD aims to improve understanding of how links between C and nutrient cycling control C storage in soils and their responses to global change. This will be achieved through the carefully designed series of experiments described in the following chapters.

1.5. MAIN RESEARCH AIMS

The overall project aim was to investigate the impact of climate change on terrestrial C storage potential. The effect of warming and nutrient availability on soil C fluxes will be assessed to investigate potential effects on soil C storage.

- A1.** To quantify the response of different below-ground respiration flux components and ecosystem processes to factorial levels of nutrient additions.

- A2.** To corroborate the influence of mixed-forage grassland management, including changing legume abundance, on the soil C fluxes and decomposition rates.

- A3.** To determine how nutrient availability affects ecosystem C-cycle response to soil warming.

CHAPTER II

THE IMPACT OF NUTRIENT ADDITIONS ON DECOMPOSITION RATES AND THE LINKS BETWEEN PLANT AND SOIL PROCESSES IN TWO SEMI-NATURAL GRASSLANDS

2.1. INTRODUCTION

2.1.1. Research questions

Understanding the linkages between plant and soil processes is essential for predicting the responses of terrestrial ecosystems to global change. Extensive consideration has been given in the last few decades to the impact of increasing reactive atmospheric nitrogen (N) deposition on terrestrial carbon (C) processes. Evidence gathered thus far shows that the relatively low soil fertility in some ecosystems has been altered by anthropogenic N enrichment (Carroll *et al.*, 2003, Morecroft *et al.*, 1994) with consequences for plant diversity and soil processes (Lee and Caporn, 1998). However, the full extent of the implications on the soil C fluxes remains unknown.

Some responses to N additions have been previously investigated in ecosystems as forests (De Vries *et al.*, 2006, Janssens *et al.*, 2010, Waldrop *et al.*, 2004), heathlands (De Vries *et al.*, 2009, Evans *et al.*, 2006), bogs and wetlands (Bragazza *et al.*, 2006), arid and tropical ecosystems (Cusack *et al.*, 2011), arctic and alpine ecosystems (Hartley *et al.*, 2010, Mack *et al.*, 2004,

Phoenix *et al.*, 2003b, Weintraub and Schimel, 2003) and agro-ecosystems (Jenkinson *et al.*, 1990, Wofsy, 2001). Such studies have determined the impact of N additions on decomposition (Craine *et al.*, 2007, Hartley *et al.*, 2010), soil CO₂ efflux and C sequestration (De Vries *et al.*, 2009, Heinemeyer *et al.*, 2007). The results demonstrated that both above and below-ground processes can be affected substantially. This effect ranges from increase C sequestration due to both a stimulation of GPP and a reduction in heterotrophic respiration in forests and heathlands (De Vries *et al.*, 2009), to C release due to a nutrient-induced increase in decomposition in arctic tundra (Mack *et al.*, 2004). Also, there is growing evidence that the positive impacts of atmospheric N deposition on C sequestration in some terrestrial ecosystems could turn negative in peatlands when more than 10 kg N ha⁻¹ y⁻¹ is accumulated (Bragazza *et al.*, 2006). However, less is known about the effects of long-term nutrient addition on C uptake in semi-natural grasslands.

Semi-natural grasslands are habitats that have not experienced improvement by agricultural ploughing, re-seeding, use of inorganic fertilisers or widespread application of herbicides (NERC, 2007), and comprise of a mixture of grasses and herbaceous plants, sedges, rushes, mosses and other low-growing species (Bullock *et al.*, 2011). There are six types of semi-natural grasslands: acidic grasslands, calcareous (or limestone) grasslands, neutral grasslands (or lowland meadows and pastures), marshy grasslands (including purple moor-grass and rush pastures), upland hay meadows and calaminarian grasslands (The Grassland Trust).

The experiments presented in this chapter were designed to bring further understanding to the effect of both N and P long-term additions on soil CO₂ effluxes in two types of semi-natural grasslands (acidic and calcareous) and give indication of the ecosystem's C uptake potential. Acidic and limestone grasslands are important components of the UK landscape covering 1 million and 60,000 hectares of the UK respectively, and having considerable conservation and amenity value. In the UK, semi-natural grasslands cover twice the area of broadleaf woodland, and have the greatest soil and ecosystem C

Chapter II

stocks per unit area after peatlands (UK National Ecosystem Assessment 2011). These two types of semi-natural grasslands contain over 300 of native plant species with an exceptional conservation value of floristic diversity (Preston *et al.*, 2002). The rapid increase in N deposition in the latter half of the 20th century has been a major contributor to UK grassland biodiversity loss (Stevens *et al.*, 2004).

2.1.2. Current perspective

Even though the severity of the impact varies greatly from one side of the country to the other, the White Peak area of The Peak District National Park has been identified as having one of the highest rates of N deposition in UK (Figure 2.1), with an excess of $3.5 \text{ g N m}^{-2} \text{ y}^{-1}$ (Phoenix *et al.*, 2003a). As a result, this area has been under continuous monitoring, with nutrient manipulation treatments also being applied for the last twenty years.

The first experiment was set up in 1990 by Morecroft *et al.* (1994) at Wardlow Hay Cop, on two representative examples of semi-natural grasslands, an acidic and a calcareous one. Research carried out on the plots established there, revealed that enhanced atmospheric N deposition can radically change the above and below-ground processes even in soil where N is not limiting plant growth (Lee and Caporn, 1998). Effects of long-term N addition on soil microbial biomass and activity have been proven to be significantly stimulated in the N-limited acidic grassland, but the complete opposite was observed in the P-limited calcareous grassland (Johnson *et al.*, 1998). On the calcareous side, Carroll *et al.* (2003) observed a massive increase in plant cover and diversity as a response to P additions, reducing the effect of the natural limiting factor.

Five years after the initial setup, additional plots were established in order to assess the effects of simulated pollutant N deposition on root-surface activities (Johnson *et al.*, 1999). Over the years, studies endeavoured to elucidate many unanswered questions such as the fate of pollutant N, by analysing the N budgets and fluxes for the two grasslands and discovering that the long-term additions have not saturated the system's capacity to mineralize nutrients (Phoenix *et al.*, 2003a). It was also demonstrated that the P additions have not resolved the limitation issues of the ecosystem; N & P continuing to be co-limiting nutrients on the calcareous grassland with only both their addition stimulating plant growth (Morecroft *et al.*, 1994).

Chapter II

Over the years, the plots were re-designed allowing further uncertainties to be elucidated. In 2005, on half of each plot the nutrient additions ceased, permitting recovery and as a result various research papers emerged. Almost two years after half of each plot was allowed to recover from treatment application, Arroniz-Crespo *et al.* (2008) reported the responses of the bryophyte community and identified P-limitation as the key factor for their loss. Later on, O'Sullivan *et al.* (2011) quantified the effects of recovery on seasonal and annual mean concentrations of soil mineral N and concluded that once intense N addition is ceased, there is significant potential for soil processes to recover too.

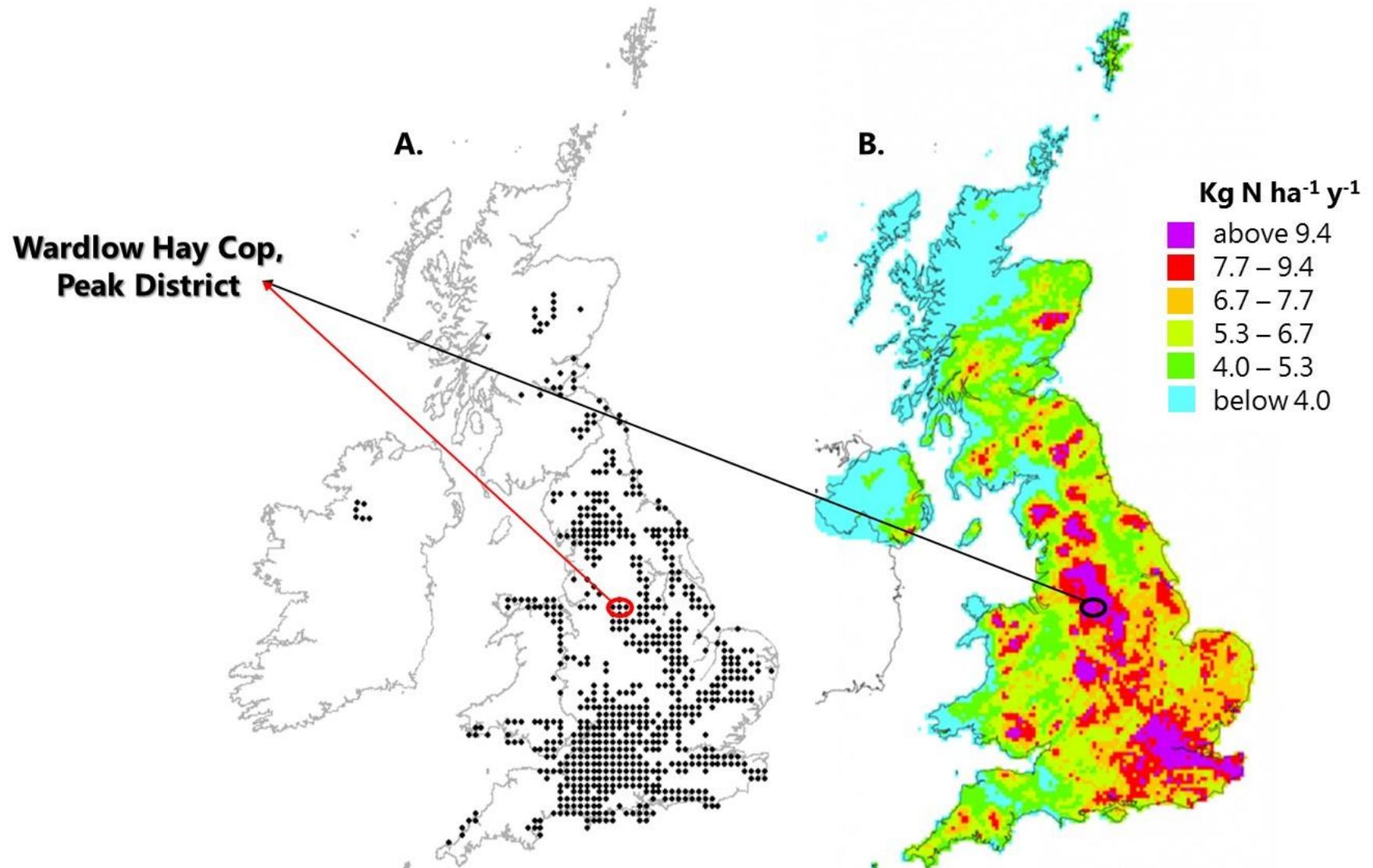


Figure 2.1. UK maps illustrating the distribution of semi-natural grasslands (A.) and areas of total oxidised N deposition (B.); highlighted in red and black are the experiments site under investigation in this chapter, at Wardlow Hay Cop in Peak District National Park (*modified from CEH & DEFRA*).

Chapter II

Continued interest in the impact of both N and P long-term additions on grassland ecosystems has generated valuable studies that improved the knowledge regarding nutrient dynamics (Phoenix *et al.*, 2004). Further clarification was brought to the N-retention potential of the calcareous grassland, where grass community maintained a low rate of inorganic N leaching to groundwater supplies (Phoenix *et al.*, 2008). Studies into the effects of different levels of nutrients additions on soil microbial community, plant nutrient accumulation and soil chemistry, indicate that even in an assumed N-saturated ecosystem, the potential for nutrient immobilisation is great (Phoenix *et al.*, 2003a), while mineral N-availability increases (Horswill *et al.*, 2008).

2.1.3. Research requirements

Grasslands in the UK cover just over half of the entire landmass, nearly three times as great as all arable crops combined. Having such an extensive distribution makes grasslands an important ecosystem, capable of sustaining and influencing key terrestrial C processes. In order to create a clearer picture of the C and nutrient dynamics in grasslands under changing climate conditions (high N deposition rates, increased temperatures, etc.), three types of grasslands were investigated in this project.

The study will start with an analysis of two semi-natural grasslands: acidic and calcareous (Chapter II), followed by improved managed grassland (Chapter III) and concluding with a laboratory controlled grassland mesocosm experiment (Chapter IV). All these experiments aim to bring together knowledge regarding interactions between C and nutrient cycling, looking at different sources of nutrients and ultimately investigating how these interactions control responses to increasing soil temperatures. In this chapter, soil CO₂ fluxes will be investigated under field conditions on a site with long-term factorial nutrient (N and P) addition treatments, established to simulate atmospheric N deposition.

Having a history of over twenty years of in-depth investigations of the N cycle, all the experiments at Wardlow Hay Cop have left at least one question to be answered: how do these treatments affect the terrestrial C cycle? Moreover, there has been little, if any, work on the site regarding soil C processes; the research so far has focused on the impacts of nutrient manipulations on vegetation distribution and diversity, making this study pertinent.

2.2. WORK AND SITE JUSTIFICATION

The work carried at Wardlow Hay Cop, Peak District set up the platform for many possibilities to explore the implications of long-term factorial nutrient additions on the soil carbon processes, which are investigated in the present chapter. On the existing setup at the site, a supplementary design was added in order to obtain data on soil CO₂ efflux, decomposition and ecosystem productivity. The main aim of this study was to expand the research already carried out on nutrient cycling to include elements of C dynamics and soil-plant interactions. The importance of this work comes from the fact that while the effect of N and P addition on ecosystem C balance has been reported in a variety of ecosystems, there is still very little known for grasslands.

Heathlands and forest studies identified a contrasting effects of different levels of nutrient addition on C sequestration (De Vries *et al.*, 2009); with difference in the relative increases of C in the soil pool versus above-ground biomass. In a tropical savannah system, Craine *et al.* (2007) detected a decrease in decomposition rates at high N availability, explained by a decrease in recalcitrant C degradability. However, in a highly N-limited ecosystem, like the arctic tundra, single nutrient additions had little effect on soil CO₂ production, in the short-term, while combined N and P additions, enhanced C mineralization rates (Hartley *et al.*, 2010). While some of these results cannot be extrapolated to other ecosystems, it is important to identify the all the factors that control the response of C sequestration to nutrient additions, such as soil type, microbial community, temperature and vegetation cover.

Wardlow Hay Cop is a unique experimental site in the UK which offers the opportunity to study more of the soil processes controlled by nutrient availability and fill some of the knowledge gaps identified. At the same time, this location is representative for a majority of ecosystems affected by intense atmospheric N deposition (Figure 2.1) and because semi-natural grasslands cover more than 16 % of the land in UK (NERC, 2007). Long-term N enhancement is an important factor influencing soil processes on grasslands, making it difficult to

confidently predict changes in ecosystem C storage, given that , unlike forests, the vast majority of ecosystem C stocks are below-ground (Phoenix and Lee, 2004). For example, it is known that in forests, N-induced inhibition of below-ground respiration is almost the same magnitude as the forest carbon sink (Janssens *et al.*, 2010), but it is not known if below-ground respiration is also inhibited by N addition to the same extent in semi-natural grasslands. On the other hand, there is much less evidence for P addition decreasing rates of decomposition, but again there is little information from grassland systems (Treseder, 2004).

2.3. AIMS AND OBJECTIVES

The purpose of the experiment described in this chapter is to elucidate some of the effects factorial nutrient additions have on ecosystem carbon fluxes in two semi-natural grasslands. The main objective are:

- O1.** To record autotrophic and heterotrophic soil fluxes and net ecosystem CO₂ exchange in order to determine the soil respiration rates and gross primary productivity in response to different levels of N and P additions.

2.4. HYPOTHESES

Two hypotheses have been formulated in order to test whether different levels of nutrient additions can affect the ecosystem's primary productivity and respiration rates, thus estimating the overall soil C sequestration capacity.

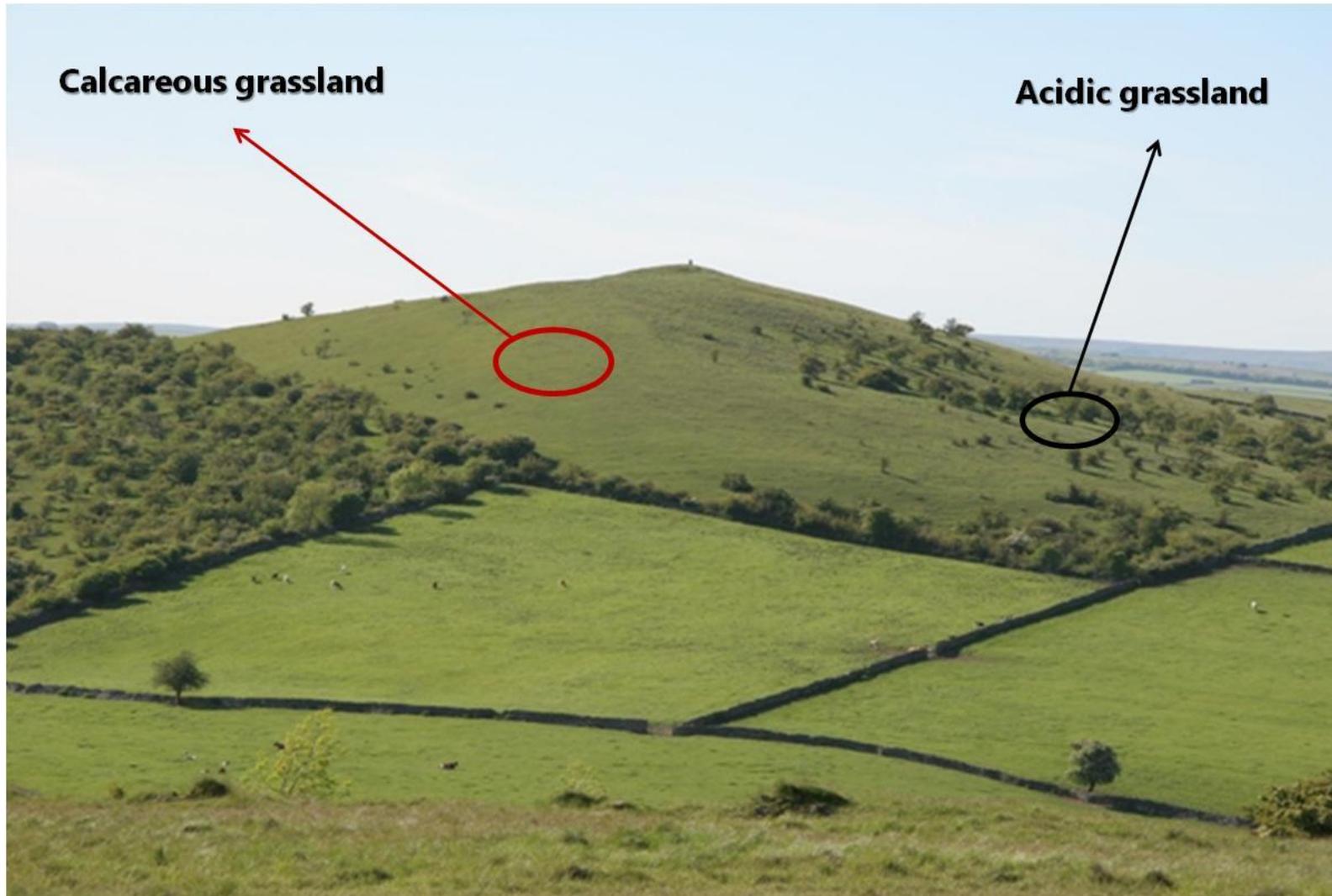
- H1.** Both inorganic N and P additions will increase ecosystem's productivity and C uptake capacity.
- H2.** The rates of decomposition (heterotrophic soil respiration) will decline, under nutrient addition, with N addition having more effect than P addition.

2.5. METHODOLOGY

2.5.1. Site description

The work was carried out at Wardlow Hay Cop (53° 16' 00" N; 370 m above sea level) in The Peak District National Park (Morecroft *et al.*, 1994). The site contains two semi-natural grasslands, a calcareous and an acidic one, situated in the Derbyshire Dales NNR, UK (NGR SK 1773) on a conical shaped hill (Picture 2.1), with underlying carboniferous limestone bedrock (O'Sullivan *et al.*, 2011). The grasslands currently experience high levels of N deposition at $2.5 \text{ g N m}^{-2} \text{ y}^{-1}$ with a past maximum value of $3.5 \text{ g N m}^{-2} \text{ y}^{-1}$ (Arroniz-Crespo *et al.*, 2008, Phoenix *et al.*, 2003a). The vegetation on the calcareous site was classified as CG2d, *Festuca-Avenula* grassland, having a shallow 5-10 cm humic *Rendzina* soil (pH 6.8) overlaying limestone. In the acidic grassland (pH 4.1), the vegetation was classified as U4e, *Festuca-Agrostis-Galium* grassland and is found where glacial loess has been deposited over limestone to a depth of 70 cm (Morecroft *et al.*, 1994). All 20ha of pasture grasslands are maintained by periodical grazing between June and December, by cattle, ewes with lambs and ponies, averaging $2.5 \text{ livestock units (LU) ha}^{-1}$ (Bilotta *et al.*, 2007). Because the plots were not fenced off, they were potentially open to the same intensity grazing.

In September 1995, both the acidic and calcareous grasslands started to receive factorial additions of N and P treatments following a randomised block design (Figure 2.2). The setup contained eighteen experimental plots of 3 m x 3 m, each with six treatments in three replicate blocks (Johnson *et al.*, 1999). Nitrogen was added as ammonium nitrate (NH_4NO_3) at the rates of 0 (control), 3.5 (low N) and $14 \text{ g N m}^{-2} \text{ y}^{-1}$ (high N) and with P added as sodium phosphate (NaH_2PO_4) at 0 (control) and $3.5 \text{ g P m}^{-2} \text{ y}^{-1}$ (low P). The treatments were applied quarterly in the first year and then monthly until November 2011. In the calcareous grassland, P additions ceased after the first year due to drastic change in vegetation biodiversity, while N continued to be added as before. In January 2005, every plot was split in two allowing one half to recover while the other continued to be treated monthly (Arroniz-Crespo *et al.*, 2008).



Picture 2.1. Wardlow Hay Cop experimental site, in the Peak District National Park, illustrating the location of the two semi-natural grasslands: calcareous and acidic (modified from the DEFRA website).

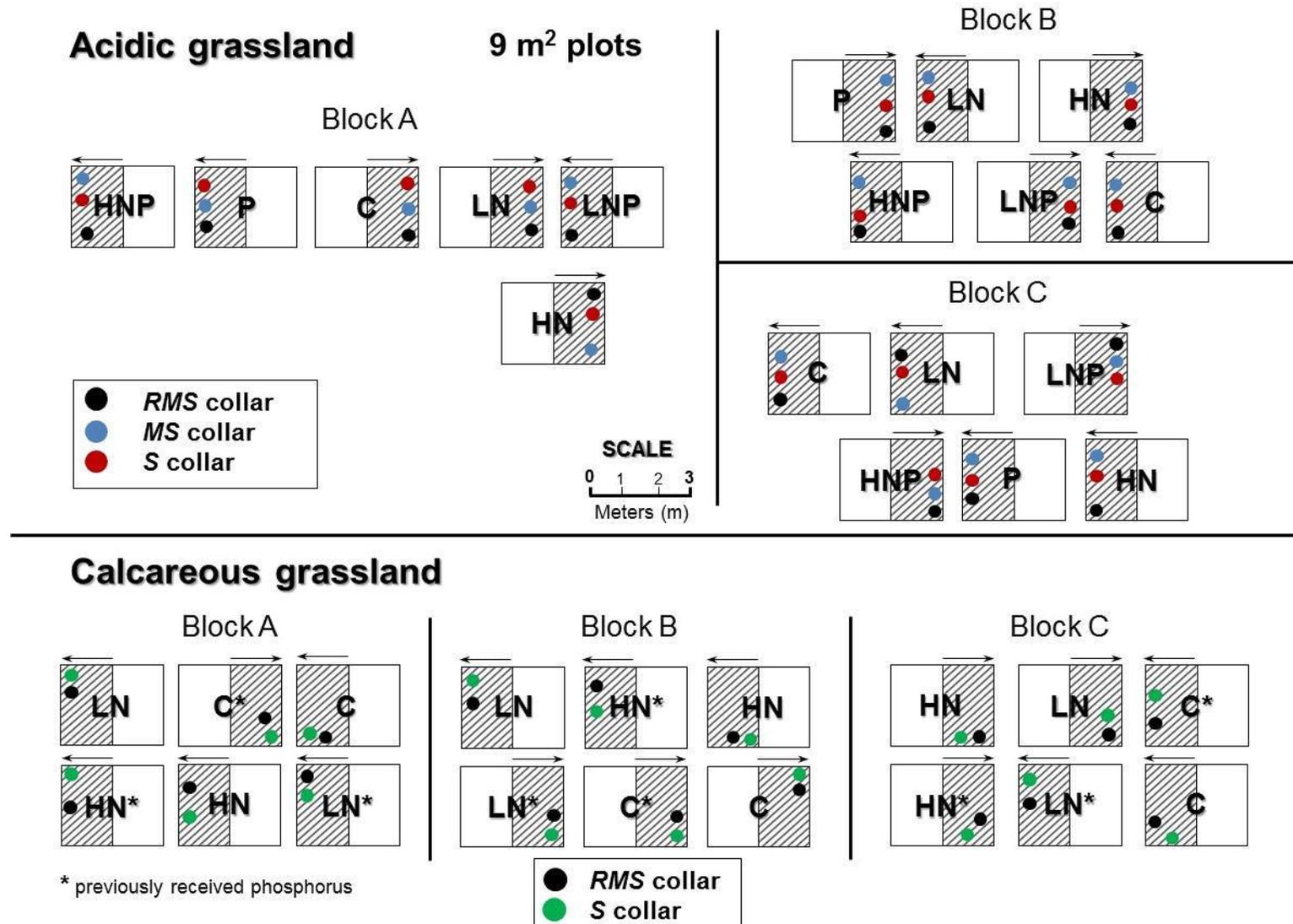


Figure 2.2. Collar position in each of the two grasslands; the hashed side of each plot represents the continuation of nutrient treatments and the blank side characterising the recovery half. Each of the blocks: A, B, C represents three replicates for all six nutrient additions. Collar type (RMS, MS and S) order was randomised in each plot.

2.5.2. Experimental design and data collection

The data collected at Wardlow Hay Cop, Peak District from both semi-natural grasslands included net ecosystem CO₂ exchange, soil CO₂ fluxes, soil and air temperature and moisture. These measurements were recorded during a five-month period in 2011, from May to September. Due to time, finances and staff availability to access the site, only four visits were made to collect the data. Nonetheless, these four sampling sessions spanned from spring, across the majority of the growing season and into autumn.

In order to test the hypotheses it was important to take measurements on the half of the plot that had no interruptions in the long-term nutrient addition treatments, thus making the recovery half less relevant for this study. Therefore, resources were dedicated to establishing flux monitoring in the long-term nutrient manipulation plots, with this still requiring the establishment of 108 soil respiration collars.

2.5.2.1. *Soil temperature and moisture measurements*

Following the soil flux measurements, soil temperature and moisture were measured during every session, by inserting the probes approximately in the middle of each collar. The temperature was assessed at three different depths (2 cm, 5 cm & 8 cm), measured using a thermometer (Salter Gourmet Digital Meat Thermometer, Tonbridge, UK), while the moisture was assessed at 5 cm depth using a 4-pin moisture probe (ThetaProbe Soil Moisture Sensor - ML2x, Cambridge, UK).

2.5.2.2. *Soil respiration measurements*

The soil CO₂ fluxes measured in the field were partitioned into 'autotrophic' components, incorporating roots and mycorrhizal fungi (AM – arbuscular mycorrhiza), and heterotrophic components, using an established mesh-collar approach (Figure 2.3), modified from the version used by Heinemeyer *et al.*

(2007). This design required a total of 108 collars (36 shallow and 72 deep) which were built using 16 cm diameter PVC pipe (Plumb Centre, Wolseley UK, Ripon, UK). The shallow collars were cut 6 cm long and were fitted in the field by lightly pressing them down into the ground, approximately 1 cm deep (Figure 2.3). In order to prevent air leakages when the CO₂ flux measurements were made, each shallow collar was sealed on the soil surface using putty. The deep collars, cut 15 cm long, were inserted 10 cm into the soil severing the roots.

The fluxes measured at the shallow collars were compared with those from collars inserted deeply into the soil, severing roots. The aim was to establish two types of deep collars in both grasslands, with the two types of collar having different aperture meshes fitted to the windows. One half of the deep collars received 20 µm meshes, allowing hyphal in-growth to take place, while the other half with 1 µm meshes excluded any mycorrhizal colonisation. Above-ground biomass was removed from all collars by frequent clipping allowing only below-ground respiration to be measured.

Because of the difference in soil profile depth and bedrock height on the two types of grasslands the collar design had to be altered. The acidic grassland had a deep soil profile, allowing all three types of collars (**a.**, **b.**, **c.**) to be deployed without interferences. Whereas, in the calcareous grassland the shallow soil profile and rocky surface prevented the successful setup of three types of collars without damaging the meshes and thus compromising the experiment. For this situation, in the calcareous grassland the deep collars were inserted without having any mesh windows and as such only one deep collar was required per plot, in addition to the shallow one (**d.**, **e.**).

Thus, for each of the eighteen treatment plots in the acidic grassland, a set of three collars was deployed as follows:

- a. RMS** – 6 cm long shallow collars, measuring the respiration of roots, mycorrhizal fungi and soil decomposers;

Chapter II

- b. **MS** – 15 cm long collars with 20 μm mesh windows, measuring mycorrhizal fungi and soil decomposers;
- c. **S** – 15 cm long collars with 1 μm mesh windows, allowing only soil decomposers influence.

On the other hand, the calcareous grassland received the following collars in each treatment plot:

- d. **RMS** – 6 cm long shallow collars, measuring the respiration of roots, mycorrhizal fungi and soil decomposers;
- e. **S** – 15 cm long collars without any windows, allowing only soil decomposers influence.

In May 2011 all the collars prepared were deployed in the field and the experiment began. Measurements were recorded on four different sessions: in May immediately after installation, August and twice in September.

The soil carbon fluxes were obtained using a portable infrared gas analyser - IRGA (EGM-4; PP Systems, Amesbury, U.S.A.), connected to a 16 cm diameter Perspex chamber. Three repeated measurements were taken for each collar by placing the chamber on top of the rim for 90 seconds as illustrated in Picture 2.2.

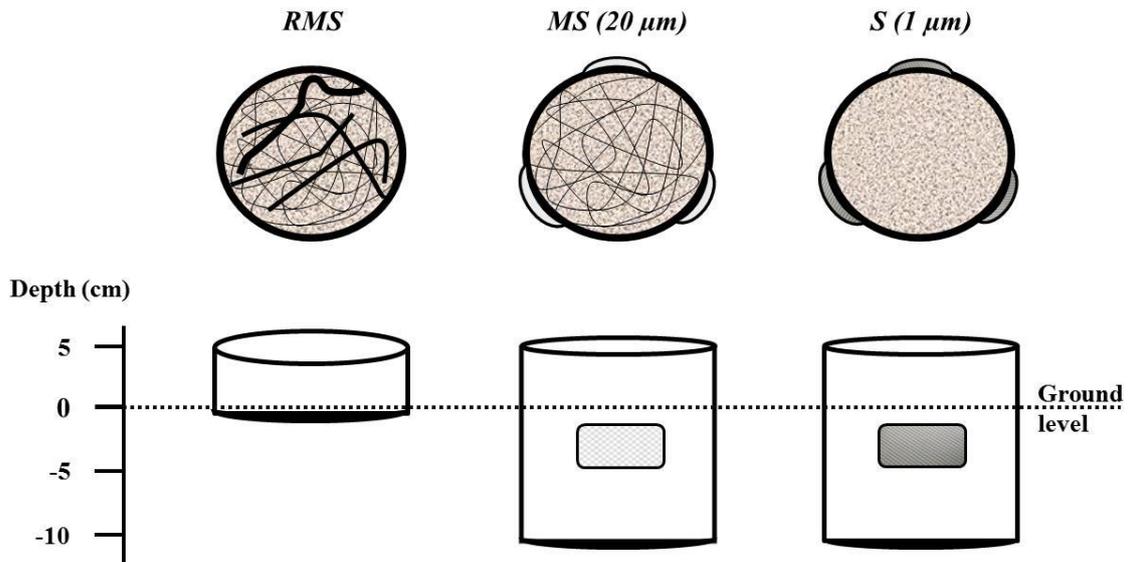
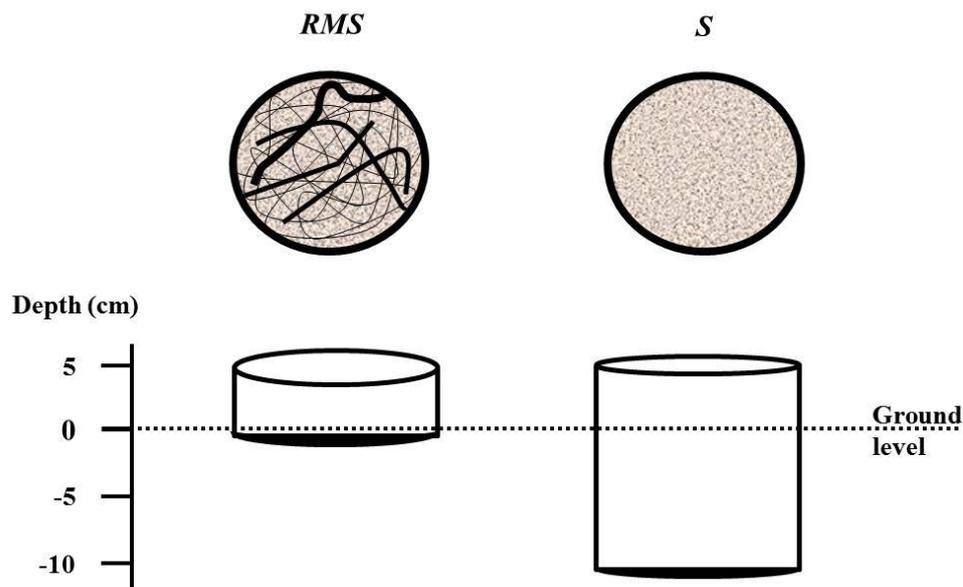
A. acidic grassland**B. calcareous grassland**

Figure 2.3. Set of collar types and insertion depth for each of the grasslands (A. – acidic grassland; B. – calcareous grassland). *RMS* - shallow collars measuring all soil respiration flux components: roots (thick lines), mycorrhizal hyphae (thin lines) and soil heterotrophs (grainy pattern); *MS* - deep collars with three windows covered with 20 μm mesh, severing and excluding the roots and allowing in-growth of mycorrhizal hyphae; *S* - deep collars with three windows covered with 1 μm mesh excluding both roots and mycorrhizal hyphal in-growth. The function of the *RMS* and *S* collars in the calcareous grassland is the same as the acidic one; the *S* collar is missing the mesh windows because its integrity would have been compromised on insertion and contact with the irregular bedrock depth in this grassland.

2.5.2.3. *Net ecosystem CO₂ exchange*

The design used to measure the net ecosystem CO₂ exchange was a modified version of the method applied by Street *et al.* (2007). Light response curves were measured on a 50 cm x 50 cm area, within the treated half of each 9m² plot. The system consisted of a base frame and a custom-built Perspex chamber (Geography Department Workshop, University of Exeter, UK). The aluminium frame base, resting on four cylindrical PVC legs 10 cm long, was fitted with a plastic skirt enclosing the volume of air between the base and the ground surface. The skirt was fixed in place by a heavy chain, preventing any exchange with the outside air. The chamber measured an area of 50 cm x 50 cm and 25 cm height and had two battery-powered fans attached to the interior walls in order to mix the air inside.

The CO₂ flux measurements were acquired by creating a closed-loop system incorporating the IRGA (Picture 2.3) to the above described setup. Light intensity during measurements was tracked with a PAR Quantum device (Skye Instruments Ltd., Llandrindod Wells, UK), placed inside the chamber. The light response curves were obtained by taking consecutive measurements at full light, three successive levels of shading and in full darkness, for each treatment plot investigated. Shading was obtained with the use of different shading cloths and tarpaulin, giving the following levels of shade: 0 % (full light), 30 %, 60 %, 90 % and 100 % (full dark).

Each measurement lasted for 90 seconds, allowing the air inside the chamber to reach ambient levels between measurements. Additional data was recorded for each plot in order to make the necessary calculations and flux corrections; these included air temperature inside the chamber, soil temperature and moisture, plus the exact height of each of the four legs, required for calculating chamber volume. The flux measurements at 100 % darkness represent ecosystem respiration (ER) and those in 0 % darkness the photosynthetic rate (GPP) at the ambient light level.



Picture 2.2. Portable IRGA connected to cylindrical Perspex chamber placed on top of a PVC collar recording soil CO₂ flux measurements



Picture 2.3. Net ecosystem CO₂ exchange measurements setup, including the Perspex chamber, battery, IRGA and the PAR Quantum device

2.5.3. Calculations and statistical analysis

Statistical analyses were carried out using SPSS (IBM® SPSS® Statistics 20). All soil temperature, moisture and flux data were initially processed using Microsoft Excel (Microsoft Office Professional Plus 2010, ©2010 Microsoft Corporation), where the necessary corrections were made and the data encoded and ready for statistical analyses. The graphs and figures were created by handling the data in Excel. The data was tested for normality in SPSS and then subject to Mauchly's test of sphericity and Levene's test of equality, followed by post-hoc tests. The effect of factorial treatments on collar CO₂ fluxes was tested for significance using ANOVAs. One-way ANOVA was used to test the significant difference between flux components in each treatment plot for all four sampling sessions, separate ANOVAs and repeated measurements two-way ANOVAs with collar treatment and time as the different factors. The interaction effect between the factorial additions of N and P at each collar was tested by two and three-way ANOVAs at $p < 0.05$.

Using the data collected from all three collar types it was possible to calculate the contribution of autotrophic and heterotrophic components to the soil CO₂ fluxes in each grassland. In the acidic grassland having three types of collars meant that different components of the soil CO₂ flux could be calculated, including arbuscular mycorrhizal respiration (AM). This was intended to be achieved by making the difference between the fluxes from *MS* and *S* collars ($AM = MS - S$). However, AM respiration proved not to be detectable, with no significant difference between *MS* and *S* collar respiration. Because the fluxes measured at the two deep collars did not differ significantly, a decision was made to average them. Thus the two below-ground flux components detected were calculated as:

[Eq.4] Heterotrophic respiration (**soil decomposers**) = mean (*MS* & *S*)

[Eq.5] Autotrophic respiration (**roots + AM**) = *RMS* – mean (*MS* & *S*)

In the calcareous grassland the modified design allowed the autotrophic and heterotrophic respiration components to be calculated as above, without having to calculate the average between the two deep collars, thus:

$$[Eq.6] \quad \text{Heterotrophic respiration (soil decomposers)} = S$$

$$[Eq.7] \quad \text{Autotrophic respiration (roots + AM)} = RMS - S$$

In order to assess the percentages of each respiration component the following equations were used:

$$[Eq.8] \quad (\text{Root} + \text{AM}) \% = ((RMS - \text{mean}(MS, S)) / RMS) * 100$$

$$[Eq.9] \quad \text{Soil decomposers} \% = ((\text{average}(MS, S) / RMS) * 100$$

Photosynthesis data were corrected to chamber height and volumes and areas were determined. Similar to Street *et al.* (2007), the light response of net CO₂ exchange or net ecosystem productivity (NEP), was modelled as a rectangular hyperbola given by the following formula:

$$[Eq.10] \quad \text{NEP} = \text{ER} - \frac{P_{max} * I}{k + I}$$

Here, P_{max} is the rate of light saturated photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), k is the half-saturation constant of photosynthesis ($\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$), I is the incident PPFD ($\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$) and ER is ecosystem respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). The light response curve of GPP was produced by subtracting ER from the above formula, resulting in:

$$[Eq.11] \quad \text{GPP} = \frac{P_{max} * I}{k + I}$$

The *Solver* formula in Microsoft Excel was used to help fit the data and maximise the R² value by varying the parameters ER, P_{max} and k . This gave the equation which was used to calculate GPP for an irradiance of 800 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. Seasonal changes in GPP₈₀₀ and ER were investigated using repeated measurements ANOVA in SPSS, with treatment type as the between-subject factor and the date of measurements as the within-subject factor.

2.6. RESULTS

2.6.1. Acidic grassland

2.6.1.1. *Soil temperature and moisture*

The soil temperature and moisture data (summarised in Table A-2.1, in Appendix) show general uniformity throughout the site for each individual sampling session. Statistical analysis indicates no significant difference in temperature between treatments or collar type during the growing season, even though overall higher values were recorded during the summer. On the other hand, moisture data confirmed observations made in the field and expressed a significant difference between collars, with lower values for the MS and S. Moisture also seemed to be significantly influenced by the presence or absence of N (no-N ↔ low-N: $p = 0.021$; no-N ↔ high-N: $p = 0.023$), regardless of its concentration level (Figure A2-1 and A2-2, in Appendix A).

Each separate sampling session registered very little if any correlation between soil temperature and flux or between soil moisture and flux (Figure A2-2, in Appendix A). Due to the initial disturbance caused by the collar insertion in May, the recorded data in this sampling campaign cannot be fully correlated with the rest of the measurements.

2.6.1.2. *Soil CO₂ fluxes*

Overall results indicate that below-ground respiration rates were consistently lower in the plots receiving high levels of N, for all three collars (Figure 2.4). The same was valid, whether P was present or not, although marginally higher respiration rates ($p = 0.068$) were measured when P was applied in addition to low N, compared to its combination with high N (Figure 2.4 – e, f, g).

Marginally higher soil CO₂ fluxes were recorded on the first campaign in May, due to the initial disturbance made to the soil on collar insertion. However, this

does not nullify the value of these records in the timeline; a gradual decreasing trend is observed for the other three campaigns. As expected, during the growing season, the fluxes declined following the initial disturbance, stabilising during the summer months and were lowest in autumn due to decreases in above and below-ground activity (Figure 2.4). In May, very little variation was observed between treatments. Nonetheless, the fluxes in high N plots were significantly lower ($p = 0.022$) in the absence of P addition, while no collar effect was yet detected.

In August, respiration rates were also lower in the plots receiving high N inputs, in the absence of P (Figure 2.4). No significant effects of P addition were observed. In addition, the collars become established and significant differences in soil fluxes were obvious between collars: $RMS \leftrightarrow S$ ($p = 0.027$) and $RMS \leftrightarrow MS$ ($p = 0.019$). However, no mycorrhizal respiration was detected from calculating the difference between the two deep collars. For the last two sampling campaigns in mid and late September, the differences between the collar treatments became even clearer ($p < 0.001$). During the mid-September campaign, the fluxes were significantly lower ($p < 0.045$), but only in the plots receiving both and P additions. Throughout, there was no significant difference detected between the two deep collars.

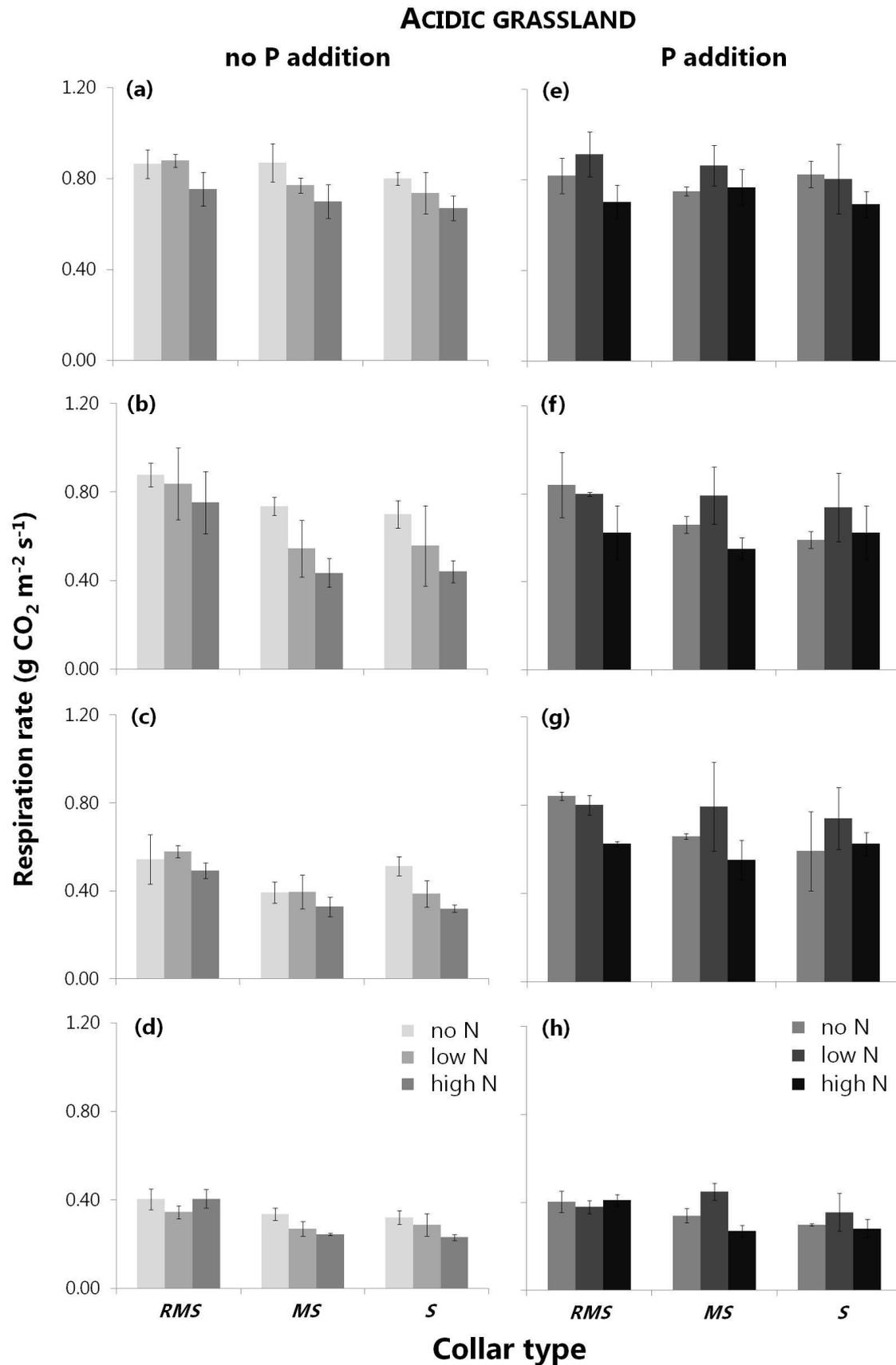


Figure 2.4. Soil respiration rates in the acidic grassland. CO₂ fluxes recorded for each collar type: *RMS*, *MS*, and *S* in May (a, e), August (b, f), early September (c, g) and late September (d, h). Data are presented by nutrient treatment (with and without P present). Error bars are \pm SE (n=3).

2.6.1.3. *Heterotrophic and autotrophic respiration*

The three collar types deployed in the field were meant to help achieve a clear partitioning between the three types of below-ground respiration: free living microbes, mycorrhizal fungi and roots. Calculations revealed that the main contributor to the overall soil respiration rate was the heterotrophic component (Figure 2.5.). Soil decomposers respiration contributed over 80 % of the flux on each sampling session, while the autotrophic components added less than 20 % (Figure 2.6).

Heterotrophic respiration recorded the highest value in May (93.11 %). There after it dropped and stabilised at 80.41 % in the summer, but gradually increased again in mid - September to 81.75 % and finally 83.19 % at the end of the same month (Table 2.1).

Opposite to the heterotrophic respiration, the autotrophic components displayed the lowest value on the first sampling campaign (6.89 %), after which it tripled in summer (19.60 %), and finally dropped in autumn from 18.25 % to 16.81 % at the end of the experiment.

Table 2.1. The rate of respiration calculated for the autotrophic components: roots and mycorrhizal fungi and the heterotrophic component during all four sampling sessions.

Sampling session	Autotrophic respiration %	Heterotrophic respiration %
May	6.89	93.11
August	19.60	80.40
September - mid	18.25	81.75
September - late	16.81	83.19

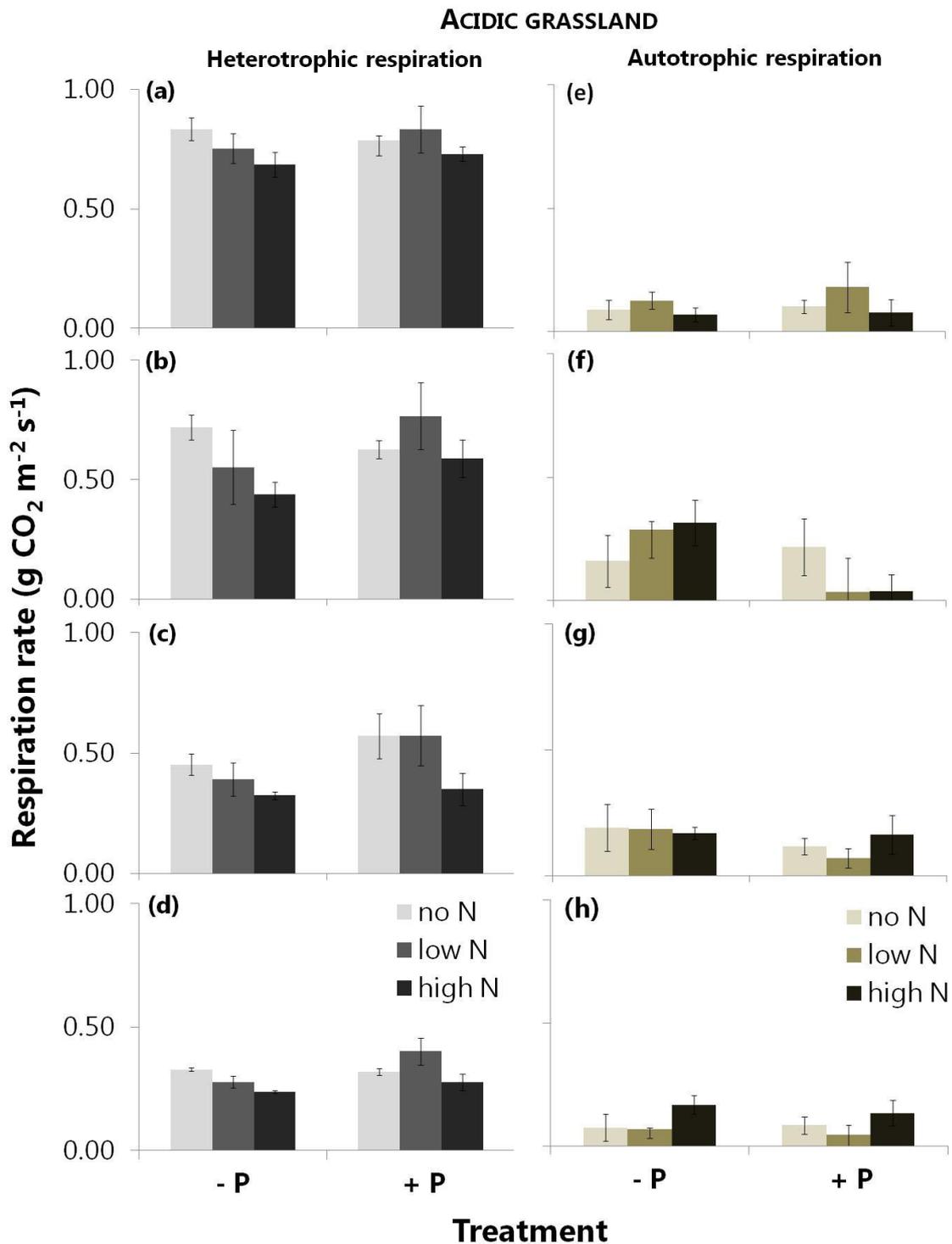


Figure 2.5. Heterotrophic (grey bars) and autotrophic (brown bars) respiration rates calculated for the acidic grassland. Fluxes are grouped by plots with and without P addition. The lighter bars represent no N addition, followed by darker bars for low N addition and the darkest bar for high N addition. Error bars are $\pm 1\text{SE}$ ($n=3$).

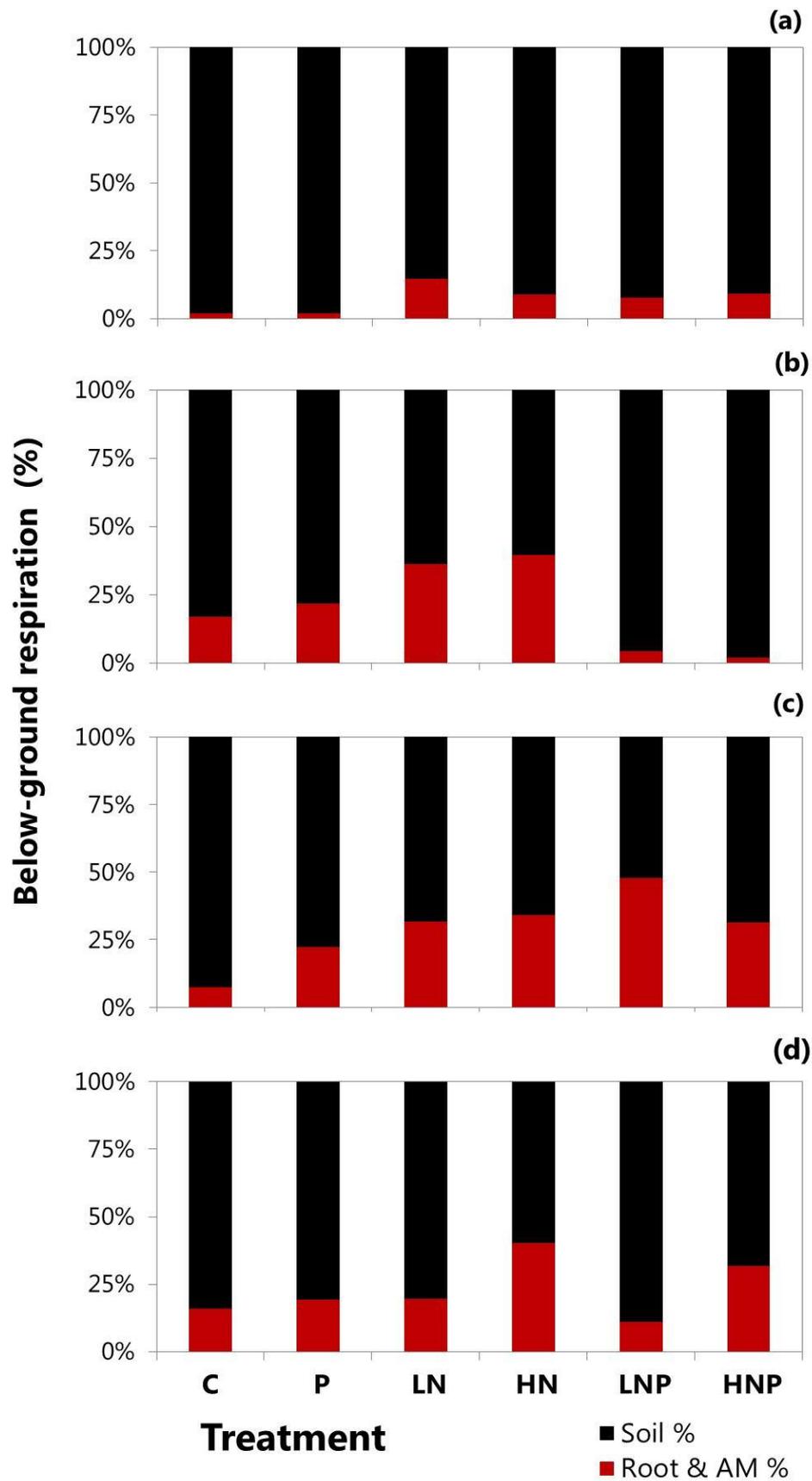


Figure 2.6. Relative contribution of root and arbuscular mycorrhizal fungi (black bars) and soil (red bars) respiration in the acidic grassland. Bars represent the total percentage of below-ground respiration in May (a), August (b), mid-September (c), and late September (d).

2.6.1.4. Ecosystem productivity

Unsuitable weather conditions, allowed for only four (C, P, HN, HNP) of the six treatment plots to be measured for net ecosystem CO₂ exchange (Figure 2.7). During the first campaign (August) the light response curves indicated slightly higher GPP at 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the plots receiving high N treatment. However, as expected, this changed towards the end of the growing season, on the second campaign (September), when both the air and soil temperatures dropped significantly ($p < 0.05$).

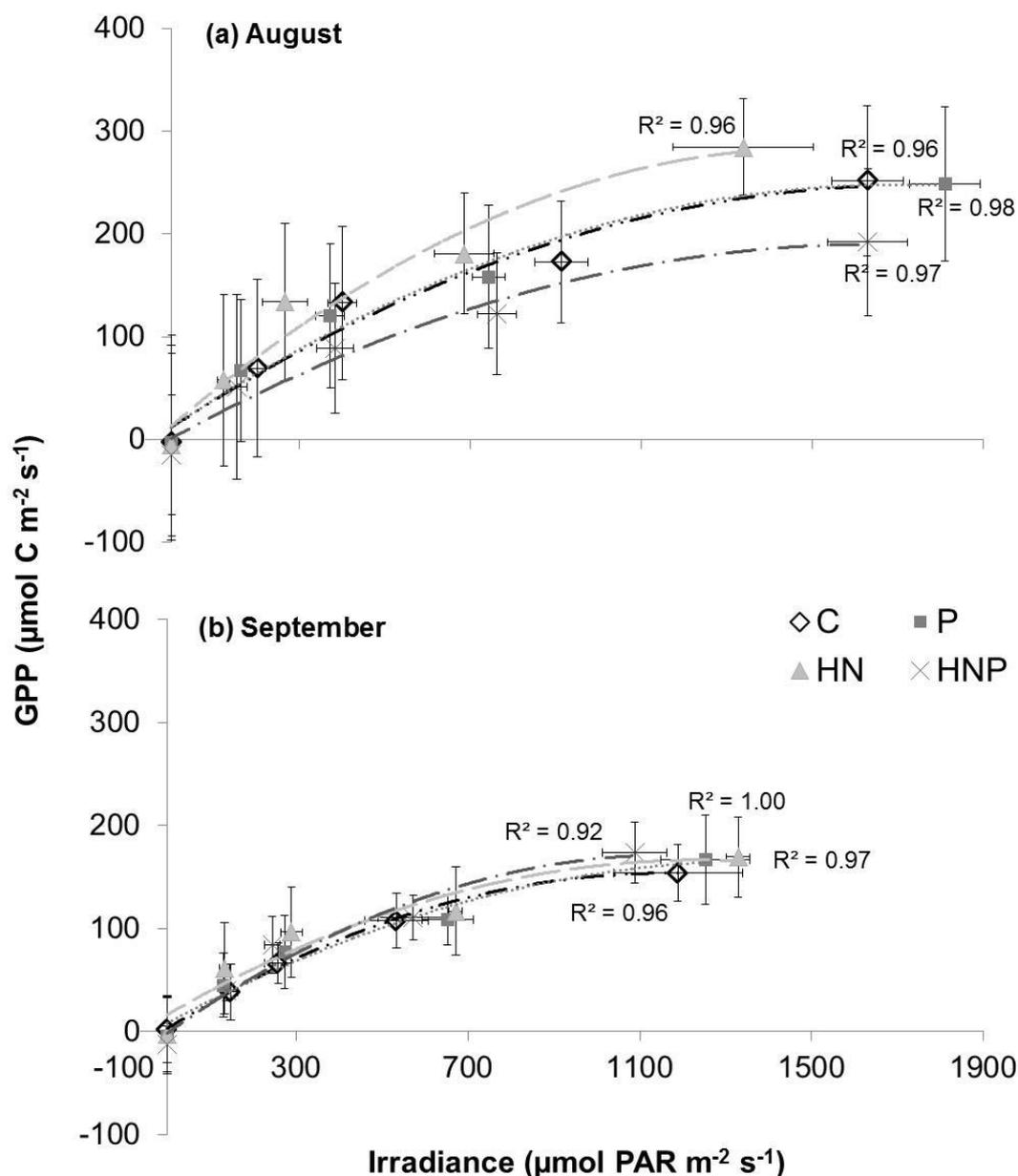


Figure 2.7. Light curves measured in the acidic grassland, on four of the investigated treatment plots (C, P, HN & HNP) in August (a) and September (b). Error bars are ± 1 SE (n=3). The parameters used in fitting these lines are shown in Table 2.2.

Table 2.2. The parameters used in fitting the lines in Figure 2.7, for all four treatments investigated (C, P, HN, HNP) during the sampling sessions in August and September.

Parameter	Treatment	Sampling session	
		August	September
ER	C	200.04	112.78
	P	164073	109.26
	HN	221.39	123.30
	HNP	155.46	121.86
P_{\max}	C	479.69	268.27
	P	370.69	260.75
	HN	430.35	205.91
	HNP	27905.62	71592.65
k	C	2187.28	951.93
	P	1037.06	776.49
	HN	734.16	380.50
	HNP	202894.46	440568.45

During the August measurements, both the rates of GPP and ER were significantly lower ($p < 0.05$) in the P-addition plots, but only where N was present (Figure 2.8 – a, c). Whereas in September, there was no significant difference ($p < 0.05$) between any of the GPP and ER data recorded (Figure 2.8 – b, d). GPP was significantly lower in September compared to August measurements in the plots receiving P treatment only and for those with high N on its own. For the plots where P was added as well as N, there was a marginal significant difference ($p = 0.108$) in GPP compared with the control plots, between the two sampling sessions. ER was significantly lower in September for the control and high N plots, with non-significant changes over time in the plots receiving P.

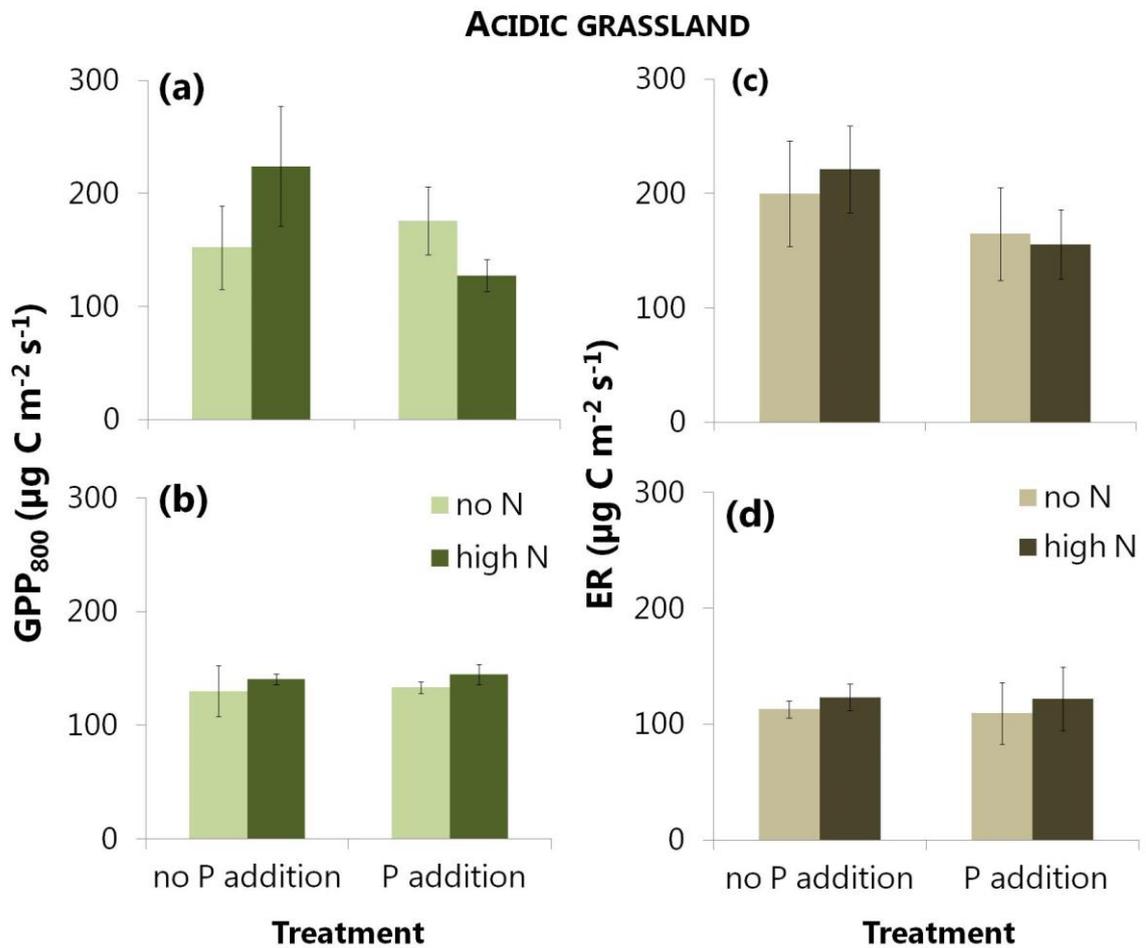


Figure 2.8. Gross primary productivity (GPP) and ecosystem respiration (ER) in the acidic grassland calculated from the net ecosystem CO₂ exchange data collected in August (a, c) and September (b, d). Error bars are presented as $\pm 1\text{SE}$ (n=3).

2.6.2. Calcareous grassland

Due to time constraints and unfavourable weather conditions, CO₂ flux measurements and background data, on the calcareous grassland, were taken only on three sampling campaigns, during May, mid and late September. Unfortunately, due to rainy and windy conditions it was not possible to collect any data on net ecosystem CO₂ exchange and productivity.

2.6.2.1. Soil temperature and moisture

The soil temperature and moisture analysis showed a significant difference between both values recorded for each treatment level and the interaction between factors ($p < 0.004$). The same was true for the repeated measurements data. On the other hand, there was no correlation between the soil temperature and moisture and the recorded flux, even when the initial disturbance represented in the May data was removed; r^2 remained less than 0.2 (Figure A-2.3 in Appendix A).

2.6.2.2. Soil CO₂ fluxes

In contrast to the acidic grassland, the results of the calcareous grassland data analysis show significantly higher fluxes for the *S* collars than the *RMS* ones. This might be attributed to the conditions in which the fluxes were measured and the physical positioning of this grassland rather than an influence of a particular treatment. The side of the hill on which this grassland was located (Figure 2.1), was exposed to windy conditions, more so than the side of the acidic grassland, where existing trees and shrubbery offered some buffer. Although the fluxes show a similar trend throughout all sampling campaigns, it is considered the problems encountered with the measurements mean that it is not possible to quantify the effects of the different treatments.

The recorded soil CO₂ fluxes were three times greater in the calcareous grassland than the acidic grassland. On all sampling occasions there was a

significant difference in respiration rates between the two collar types ($p = 0.008$), except immediately after insertion in May (Figure 2.9). Initially, higher respiration rates were observed for the plots receiving high N, in contrast with the controls, but this difference reversed later in the experiment with high N plots showing significantly lower respiration rates than both control and low N plots. Past P additions to half of the plots had marginally ($p = 0.058$) influenced the soil respiration rates, by lowering heterotrophic respiration in the high N plots in September, and recording insignificant difference between treatments in the other months.

Contrasting with the acidic grassland, the seasonal variation of the soil CO₂ fluxes in the calcareous grassland showed no clear trend (Figure A-2.4, in Appendix A). There was no evident decline from the initial record towards the end of the growing season; but, surprisingly, the fluxes seemed to decrease in mid-September, and then rose again towards the end of the month.

2.6.2.3. *Heterotrophic and autotrophic respiration*

Respiration rates were greater at the S collars than the RMS collars and therefore the autotrophic respiration calculated for the calcareous grassland presented negative values, making it difficult to interpret the results. The partitioning approach was not successful in this very exposed ecosystem.

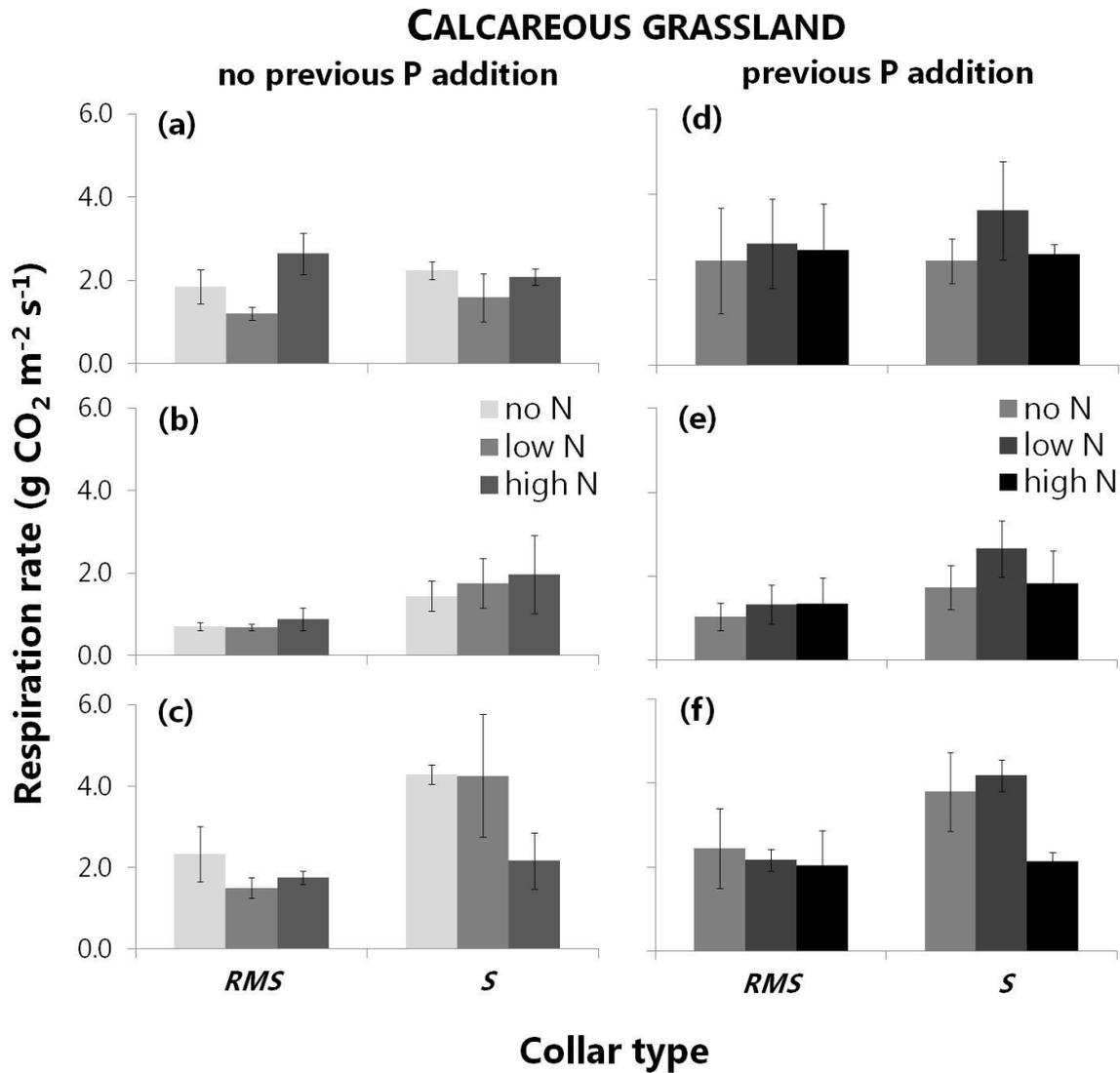


Figure 2.9. Respiration rates in the calcareous grassland, recorded for both collar types (RMS and S). Data are shown separately for the treatment receiving different levels of N only (a, b, c) and those who previously received P treatment (e, f, g), but has ceased after a couple of years after initiation. The graphs illustrate measurements taken in May (a, d), August (b, e) and September (c, f). Error bars are presented as $\pm 1SE$ ($n=3$).

2.7. DISCUSSION

2.7.1. Soil carbon fluxes and productivity in an acidic semi-natural grassland

The investigation into the C dynamics of two semi-natural grasslands has revealed dissimilar results. The initial formulated hypotheses were only partially confirmed by the data in the acidic grassland, while for the calcareous side there was not enough conclusive evidence to either confirm or reject these hypotheses. In the acidic grassland the addition of inorganic N reduced the rates of decomposition (soil respiration) and increased productivity of the ecosystem and potentially its C uptake capacity. These findings are supported by similar results obtained from forest ecosystem research (Bowden *et al.*, 2004, Burton *et al.*, 2004, De Vries *et al.*, 2006, Pregitzer *et al.*, 2008), where simulated chronic atmospheric N deposition increased above-ground biomass and reduced-below-ground respiration. In grassland ecosystems, there is some evidence of a similar trend, although of a smaller magnitude than those recorded in forests, due to the different recalcitrance of the material composing the SOM in these ecosystems (Fornara and Tilman, 2012).

The soil respiration rates and net CO₂ exchange data obtained from the acidic grassland also suggests that the reduction in decomposition, but not productivity induced by N additions, reduced the strength of the link between these two processes. This phenomenon could indicate that N deposition has the potential to increase the C storage capacity of the grassland ecosystem, but predominantly as above-ground biomass (Manzoni *et al.*, 2012), with less incorporation into the soil matrix (Lu *et al.*, 2010). Such an increase in C storage as biomass could be driven by higher rates of photosynthesis and/or decreased C allocation to mycorrhizae (Pregitzer *et al.*, 2008). As is the case for this experiment, a large increase in N availability can reduce the need for plants to invest C in nutrient-absorbing systems, such as mycorrhizae (Janssens *et al.*, 2010). The decline of mycorrhizal root symbionts responsible for SOM

degradation could be the reason for the lack of difference in respiration rates measured from the two deep collars.

However, these effects of N additions seemed to be valid only for the plots receiving high N levels and in the absence of P. This behaviour could be the result of chemical inhibition of the microbial community (Craine *et al.*, 2007, Dijkstra *et al.*, 2006) responsible for the main part of below-ground respiration rates. Other studies too have found that N alone and in high dosage can increase soil C sequestration, while multi-nutrient treatments mainly increase productivity of the ecosystem (Fornara *et al.*, 2013). At Wardlow Hay Cop, the addition of P together with high N reduced respiration rates slightly, but only compared to the plots receiving both P and low N, with no difference compared with the control and therefore, the negative impacts of high levels of N addition on decomposition rates appeared to be reduced when P is also added.

The response of decomposition to an additional N source is also tied to the presence of labile C, which is usually preferred by microbes over recalcitrant C (Craine *et al.*, 2007, Hobbie, 2005). Decomposition and thus possibly below-ground respiration could be encouraged by N additions as long as labile C is present too. However for this experiment, the only source of labile C, at least in the beginning, would have been the severed roots dying inside the collars. Initially, the root exudates could have triggered the activity of the microbial biomass responsible for producing enzymes necessary for SOM decomposition (Fontaine *et al.*, 2004); thus, partially explaining the high respiration rates recorded at the beginning of the experiment.

The nature of the soils is also a factors that can influence the net C loss from an ecosystem; highly organic soil would be more susceptible to losing C than mineral ones (Hopkins *et al.*, 2011, Soussana *et al.*, 2004). Other site-specific factors like grassland type (newly established versus permanent) (Byrne *et al.*, 2005), N fertiliser supply (Sillen and Dieleman, 2012), grazing intensity (Soussana *et al.*, 2010) can have additional effect on the soil fluxes. Although, the most important factors perhaps are annual rainfall, temperature and

grassland management (increased fertilisation, grazing), which have carry-over effects on soil C pools (Soussana *et al.*, 2010). Thus, in a semi-natural, moderately grazed grassland, like Wardlow, receiving nutrient fertiliser in addition to the already high background N deposition, there seems to be still potential for increasing the soil C pool.

2.7.2. Soil carbon fluxes in a calcareous semi-natural grassland

On the other hand, the results from the calcareous grassland are more difficult to interpret. Strong winds and continuously lower temperatures during measurements might have affected the seal between the chamber and collar. This effect could have resulted from a negative pressure created in the chamber and facilitated the suction of more soil CO₂ than under normal pressure conditions (Fang and Moncrieff, 1998, Schoffelen *et al.*, 1997), especially from the S collars which were inserted deep into the soils and thus wind would not have caused leaks between the collar and soil surface. Thus, the CO₂ fluxes recorded from the S collars on the calcareous grassland were almost three times greater than those from the acidic site. An initial interpretation of the results revealed that there was no significant correlation between the soil fluxes and soil moisture and temperature, but there was a significant difference between the measurements and treatments. The very high fluxes in the S collars could have been influenced by the high pressure affecting the measurements from the deep collars.

Recent studies suggest that while a continued increase in N deposition might increase C storage in plant biomass, the actual accumulation in soil C storage is small (Sillen and Dieleman, 2012). For the moment the grassland ecosystem acts as a residual sink, but several mechanism responsible for the terrestrial accumulation of carbon are expected to become less effective in the future, possibly leading to a diminished sink and perhaps even an additional source. Therefore, temperate grassland soils could be forced out of equilibrium, with a potential brief increase in soil C as a result of increased net primary productivity (Soussana *et al.*, 2010). Data modelling for the period between 2008 and 2012

estimated an average C flux in European grasslands of approximately $0.52 \text{ t C ha}^{-1} \text{ y}^{-1}$ C uptake (Vleeshouwers and Verhagen, 2002). These values suggest that the European grasslands will continue to act as a C sink for the period under investigation.

2.7.3. Impacts of stochastic nutrient additions

Besides the controlled, quantified nutrient addition treatments, the plots received another type of fertiliser that is not easily quantified and doing so was out of the scope of this project. The grazers present on the site approximately six months every year provided an organic nutrient addition to some of the plots, which was not monitored or quantified. This could have made small changes to the existing experimental design and influenced some of the recorded fluxes. The activity of these animals can have a strong influence on soil physical and biological qualities especially with regards to soil structure, porosity, aeration, water infiltration, drainage, nutrient/ elemental cycling and organic matter pool and fluxes (Lal, 2004). Under intensive grazing, up to 60% of the above-ground dry-matter production is ingested by domestic herbivores (Soussana *et al.*, 2004). This was also observed on the acidic site where a change in the dominant vegetation type was observed in the plots receiving P treatment, coinciding with the presence of the grazers. There, preferential grazing of the P plots led to a reduction in grasses and forbs, and increased moss cover by removing the competition for light.

In addition to increased defoliation and excretion, grazers can induce various other types of degradation to an ecosystem. Some of these effects are the changes to rhizosphere as a result of compaction, plugging, and poaching and can further decrease biodiversity and vegetation percentage cover of the pasture (Bilotta *et al.*, 2007). Also, the already spatially variable soil C could be increased by returns concentrated in excreta patches (Soussana *et al.*, 2010). There are numerous studies reviewing these impacts and suggest that in most cases grazing can increase soil C in warm dry regions, with a long history of grazing by 7.7 %, but those not meeting this criteria could lose an average of

Chapter II

1.8 % per year (Conant *et al.*, 2001). Like any other treatment, the intensity of grazing can affect differently the soil C storage and Soussana *et al.* (2010) noticed that light grazing increased C stocks compared to heavy grazing or the enclosure area. Conant *et al.* (2001) reports that when production decreases as a result of grazing, soil C content could still potentially increase, only if offsets the reduction in aboveground biomass inputs to soil. This could also be the case in the acidic grassland where the results indicate lower respiration rates when both N and P nutrients were added to the plots.

2.8. CONCLUSIONS

The overall results confirm the formulated hypotheses, indicating that N additions can increase productivity in acidic grassland. However, there is no strong evidence that P addition increased productivity. Nitrogen additions reduced decomposition rates; therefore, the strength of the relationship between decomposition and plant productivity appears to have been reduced; plant productivity remained high despite the reduction in decomposition rates. Thus, plant productivity was less dependent on the rate at which nutrients were released during decomposition. The absolute reduction in decomposition rates, with no corresponding reduction in productivity, also indicates that N deposition has the potential to further increase C storage in acidic grasslands. Data still needs to be gathered for the calcareous grassland in order to determine whether the same effect is observed.

CHAPTER III

THE EFFECTS OF NITROGEN FIXATION ON SOIL PROPERTIES, PRODUCTIVITY AND CARBON FLUXES IN A MANAGED GRASSLAND

3.1. INTRODUCTION

3.1.1. Research questions

Human alterations to the global N cycle, including cultivation of legumes, use of N fertilisers and N released from combustion engines, produce an excess of almost twice the magnitude of N fixed by natural processes (Vitousek *et al.*, 1997). As demonstrated in Chapter II, in the acidic Peak District grassland, there is growing recognition that inorganic N additions to terrestrial ecosystems can reduce decomposition rates in soils. However, less is known about whether similar effects occur when planting legumes in intensively managed grasslands. Today, humans have the technology to fix over 40 million tonnes of N yr⁻¹ through the cultivation of legumes (Jenkinson, 2001). Enhanced biological N fixation is potentially an important management option in nutrient-limited terrestrial ecosystems, but we do not fully understand what the implications could be for net N and C balances (Rastetter *et al.*, 1997, Vitousek *et al.*, 2002).

Previous studies indicate that additions of N fertiliser to grasslands, can dramatically change plant species composition and diversity, whilst stimulating

increased aboveground C storage (Zeng *et al.*, 2010). At the same time enhanced N availability, through fertilisation or atmospheric deposition (Hartley *et al.*, 2008, Janssens *et al.*, 2010, Zeng *et al.*, 2010), causes mainly negative impacts on vegetation biodiversity and has a very important effect on the soil C pools.

Predicting how the soil C sink will respond to altered nutrient availability, due to future global change (Bradford *et al.*, 2008), might be possible by investigating the individual factors influencing the interaction between C and nutrient cycles (Sistla and Schimel, 2012). One of these factors is the biological N fixation in legume-based agricultural systems. It is long known (Hardarson and Atkins, 2003, LaRue and Patterson, 1981, Ryle *et al.*, 1979) that nodulated legumes have the potential to influence the balance and availability of N and C in the soil. This implies a potential to reduce the need for inorganic N fertilisers, but only if legume use proves to be more efficient in terms of seed and workload costs, plus the energy and resources needed by the plants to fix N₂.

There are serious impacts on public health and the environment due to heavy agricultural reliance on synthetic chemical fertilisers and pesticides (Pimentel *et al.*, 2005). Some of the advantages of lowering or even fully replacing fertilisers with N₂-fixing legumes are the potential to reduce run-off to water resources, as well as production and transportation costs, and, importantly, reduced CO₂ emissions to the environment (Sutton *et al.*, 2011). Each year in Europe, manufactured fertilisers add 11 million tonnes of N_r to fields, producing a crop growth of €20 billion to €80 billion (Sutton *et al.*, 2011). The same study reports an extra 17 million tonnes of N added by biological fixation and recycled sources with a total direct benefit of €25 billion to €130 billion.

As the more effective source of nutrients, the capacity of legumes to fixate N, could also be the answer to improving plant nutrient use efficiency, and reducing the run-off to surface water. The benefits of a clean fertilisation source and the potential to maintain good surface waters quality, makes the biological N fixation an avenue worth further investigation. Previous studies have

identified that a mix of management practices and nutrient sources could help reduce the run-off to surface waters and overall improve the nutrient use efficiency of plants (Baligar *et al.*, 2001, Mitsch *et al.*, 2001). However, further research is required to determine the perfect balance between inputs and outputs.

Rising economic (energy) and environmental (pollution) costs of manufacturing, transporting and using industrial N-fertiliser, make the exploitation of biological N₂ fixation in agriculture increasingly attractive and potentially mandatory during the next century. The amounts of N fixed from atmospheric N₂ in legume/grass pastures throughout the world ranges from 20 to 200 kg N ha⁻¹ yr⁻¹ (Ledgard and Steele, 1992) with a potential between 200 to 400 kg N ha⁻¹ yr⁻¹ for a wide range of legumes. Crews and Peoples (2004) advocate a greater use of legumes for N and food supply, suggesting the legume-based systems may be more sustainable than fertiliser-based systems.

3.1.2. Current perspective

Inorganic N additions on the acidic grassland in Peak District resulted in the reduction of heterotrophic soil respiration, while the plant productivity was not affected by the rates of nutrient release during decomposition. At the same time, the strength of the link between plant and soil processes was controlled by the long term nutrient additions of both N and P. More importantly, the absolute reduction in decomposition rates, with no corresponding reduction in productivity, also implied the potential for soil C storage increase, due to N deposition.

Total C storage in ecosystems is controlled by the balance between production and decomposition (Mack *et al.*, 2004) and is relatively insensitive to single nutrient availability (Hartley *et al.*, 2010). However, any N additions can lead to significant changes to the rate of CO₂ released from soils (Gray and Fierer, 2012). Research carried out by Bowden *et al.* (2004) indicates that initial N addition to a terrestrial ecosystem, increases soil respiration, by enhancing the photosynthesis and stimulating the increase in biomass. After the initial year of continuous fertiliser addition, regardless of its volume, the respiration reaches a neutral point, and gradually decreasing thereafter as evidence of stabilisation. Similar results were also generated in a meta-analysis of temperate forest responses to N deposition; rates of decomposition declined leading to reduced rates of ecosystem respiration per unit ecosystem productivity under high rates of N deposition (Janssens *et al.* 2010).

However, there have been fewer studies considering the effect of N fixed by legumes on ecosystem C storage (Conant *et al.*, 2001, Li *et al.*, 2011, Sorensen *et al.*, 2012). Research indicates that N₂ fixation rate in many ecosystems is driven by factors relating to global change, like warming and increased N deposition, that could cause continued C losses from organic matter, accumulated in the past, due to accelerated microbial activity (Sorensen *et al.*, 2012). The introduction of legumes can increase soil N, resulting in superior soil fertility, further increasing aboveground and belowground production (Conant *et*

al., 2001). In addition to increasing forage production, combining grasses and legumes often results in increased belowground production. This in turn could lead to greater total plant–soil system C, and thus the potential to sequester more C from the atmosphere (Conant *et al.*, 2001, Crawford *et al.*, 1996).

In this context litter decomposition is an important process to study, as associated nutrient mineralization, plays a key role in controlling ecosystem productivity (Knops *et al.*, 2001). There is evidence of greater retention of both C and N in the fields where legumes are present, indicating that legumes may lower decomposition rates, especially those with low C-to-N ratio residues maintain soil fertility and potentially reducing pollution of waterways (Drinkwater *et al.*, 1998). This effect has the potential to restore the biological linkage between C and N cycling, and could lead to improved global C and N balances. Thus, it is important to improve understanding of how the presence of legumes affects decomposition rates of litters with contrasting C-to-N ratios.

This incomplete understanding of the biological N₂ fixation effects on the soil C storage and decomposition rates leaves the opportunity for further exploration. In depth understanding of the processes linking soil potential to sequester C with legume cover in grasslands can elucidate the importance N₂ fixation has on terrestrial C cycle and thus provide further information on how links between C-N cycles control patterns of C storage.

3.1.3. Research requirements

Field experiments indicate that reduced dominance of some legume species and the disappearance of others are forced by drought, pests or disease and additional inorganic N enrichment (Ledgard, 2001, Sorensen *et al.*, 2012, Zeng *et al.*, 2010). Exposure to extreme temperature events, especially in environments with high light intensity, substantially damages the N-fixation capacity, which suggests that predicted increases in the frequency and intensity of extreme temperature events in the next century (IPPC, 2007) could reduce biological N inputs in some environments. This could, in turn, lead to increased fertiliser N inputs to satisfy the higher N requirements of a more productive vascular plant community in warmer climates (Gundale *et al.*, 2012). Data has been gathered for some ecosystems regarding their behaviour to modifications in biological N-fixation due to global change, but not enough is known on the impacts these may have on the soil CO₂ fluxes in managed grasslands.

Pimentel *et al.* (2005) reports a soil C increase of 981 and 574 kg ha⁻¹ in organic legume systems, compared with only 293 kg ha⁻¹ in the conventional system. Nyfeler *et al.* (2011) found that soil N availability increased in grass-legume mixed sward compared to monoculture pastures and this availability decreased with higher legume proportion in the mix. This fact implies that mixed sward productivity increases by transference of additional N from legumes to grasses. This suggests that even though symbiotic N₂ fixation happens exclusively due to the presence of legumes, the accompanying grasses provide important feedback enhancing the fixation mechanisms. Thus, the feedback regulation consists of N₂ fixation by uptake from the unlimited atmospheric source, according to the N demand from the sink strength of the whole sward (Nyfeler *et al.*, 2011).

While other studies (Fornara and Tilman, 2008) achieved similar results by using different combinations of functional traits in grasses and legumes, they left unanswered the question of if and how these might affect below-ground productivity and soil C accumulation.

Even though more knowledge is gathered each year, some matters regarding the behaviour of N in the environment, the links between N fixation and soil C fluxes and the potential for C sequestration require further investigation (Li *et al.*, 2011). As described above, due to relatively high potential for C sequestration rates and extensive grassland coverage, improved grassland management is potentially a substantial global sink for atmospheric C (Conant *et al.*, 2001). Thus, this chapter aims to elucidate some of the issues regarding the effects of legumes on soil CO₂ effluxes in a managed grassland.

3.2. WORK AND SITE JUSTIFICATION

The WEB (Widescale Enhancement of Biodiversity) experiment at North Wyke is part of a nationwide project funded by DEFRA investigating the fate of nutrients in the environment and better management of agricultural sites in order to reduce loss by leaching or emissions (more details on the experiment are provided in section 3.5.1). The experiments presented in this chapter started in the last year of the wider WEB project and 5 year after the initial sward was established. These factors make the WEB the ideal platform to test the effects of medium-term legume cultivation on productivity, decomposition and C fluxes.

In the five years since its commencement, the WEB field site has been under scrutiny regarding a multitude of ecosystems services, biodiversity of flora and fauna and most importantly yearly yields due to different management practices. All these studies investigate mainly the fate and pathways of N in a managed grassland system for the purpose of qualitative and quantitative foraging material production. However, little, if no research was conducted on the influence of the N₂ fixation on decomposition rates and soil C fluxes, which is receiving growing interest in the scientific community. Previous studies investigated a variety of processes relating to N and C cycles, separately, and not enough is known about the interdependent relationship between them. Also, the majority of existing data focus mainly on arctic tundra and forests at all latitudes and very little information exists on grasslands. The present chapter explores the process of N fixation by legumes in a managed grassland with implications on productivity, decomposition and soil C effluxes as an indication of the soil storage capacity.

3.3. AIMS AND OBJECTIVES

The main aim of the research carried out at North Wyke was to determine the effect N₂ fixation by legumes has on soil CO₂ fluxes and litter decomposition rates. In order to achieve this, three objectives were formulated:

- O1.** To measure soil CO₂ fluxes in replicated grassland plots, with and without legumes.
- O2.** To measure rates of decomposition of both grass and woody litter in the presence versus absence of legumes.
- O3.** To place these results in the context of changes in key ecosystem and soil properties measured by the researchers at North Wyke: grass yield, total soil C, N, P, pH and water soluble N and P.

3.4. HYPOTHESES

Meeting these objectives allowed the following key hypotheses to be tested:

- H1.** Total, heterotrophic and autotrophic below-ground respiration will be lower in the presence of legumes because of the greater soil N availability
- H2.** The decomposition rates of contrasting litter types will be lower in the presence of legumes, again, due to the greater soil N availability

3.5. METHODOLOGY

3.5.1. Site description

The experiments described in this chapter were carried out on a DEFRA funded project called the WEB experiment at North Wyke Research Centre (NW) in Devon, UK, part of Rothamsted Research. The WEB plots were set up in spring/early summer 2008 onto existing grassland, by sowing a range of legume and forb species under different management practices. The main objective was to quantify the effect of each seed mix on the productivity, quality of forage, improvement of soil structure and reduction of nutrient loss. Seventy-two plots (18 treatments x four replicates) were established using a nested randomised block design.

Three seed mixtures (Table B1-1, Appendix B) were applied in each replicate block. Half of the plots were cut and the other half experienced moderate cattle grazing at circa three livestock units ha⁻¹ (WEB, 2013). These mixtures were: grass only mix (G) comprising five species; grass + legume mix (GL) comprising five grasses and seven agricultural legumes; grass + legume + non-legume forb mix (GLF) comprising five grasses, seven legumes and six non-leguminous forbs. In addition, there was an unsown 'existing grassland' control nested within the grass only main treatment. The plots were managed under typical, rested, ploughed or minimal cultivation (Figure 3.1). Underlying soil is acid-neutral clay typical of much of the permanent grassland resource in the west of the UK.

The work described in this chapter was carried out for eight months, starting in August 2012; four years after the initial WEB plots were established. Using as a baseline the existing layout presented above, an additional setup was developed in order to meet the aims and objectives of this project. The plots chosen were G (grass) and GLF (grass + legume + forb) under TP (traditional ploughing) management. The G plot represents the control, while in the GLF

plot was observed the best establishment of legumes after four years from its commencement. Thus, after physically inspecting the site, the best contrast between results was estimated to come from these two types of management.

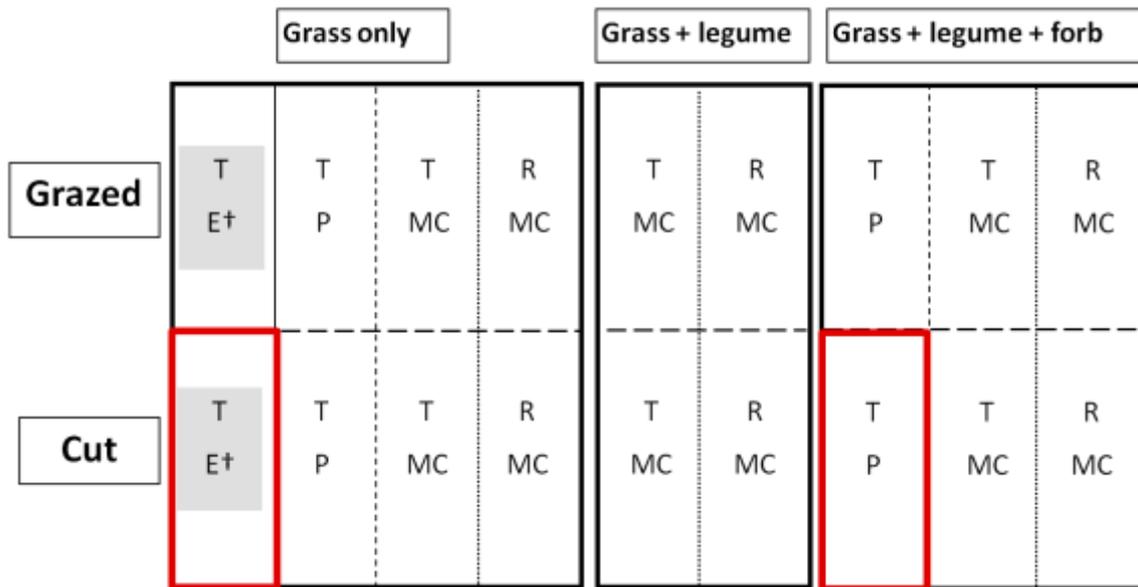


Figure 3.1. Experimental design of an example replicate block. Thick dashed line separates grazed from cut plots (by stock proof fencing); thick dotted line separates Typical (T) managed plots from Rested (R) plots (by temporary stock proof fencing); thin dashed line distinguishes plough (P) from minimal cultivation (MC) plots; the undisturbed existing grass plots in the grass only swards are indicated by T E† (no fencing between T plots). The red blocks represent the plots chosen for the experiments carried out for the purpose of this chapter



Figure 3.2. WEB experiment plots, all 72, are represented in light green and grey border. The dark green plots bordered in brown are the plots used for the experiments described in this chapter. The numbers indicate the plot ID (1 to 72), while the letters represent the sward ID (G= grass mix; GL= grass + legume mix; GLF= grass + legume + forb mix).

3.5.2. Experimental design and data collection

3.5.2.1. *Soil temperature and moisture measurements*

Soil temperature and moisture data was recorded for each collar, during every session, by inserting the probes approximately in the middle of each collar. The temperature was assessed at three different depths (2 cm, 5 cm & 8 cm), measured using a thermometer (Salter Gourmet Digital Meat Thermometer, Tonbridge, UK), while the moisture was assessed at 2 cm and 5 cm depth using a 4-pin moisture probe (ThetaProbe Soil Moisture Sensor - ML2x, Cambridge, UK).

3.5.2.2. *Soil respiration measurements*

On the eight chosen plots (4 x TP - G and 4 x TP – GLF), collars were installed to measure soil CO₂ fluxes, by partitioning the efflux into autotrophic and heterotrophic respiration. Each plot was fitted with two types of collars, similar to those used in the calcareous grassland in Peak District (Chapter II – 2.5.2). In order to reduce the variability within the plots, internal replicates were setup, thus each plot consisted of three pairs of collars. The data obtained was then averaged for each plot before statistical analysis was carried out. Thus, in total, 48 collars were used (8 x 3 shallow and 8 x 3 deep). The shallow collars were 6 cm long and were fitted in the field by lightly pressing them down into the ground, approximately 1 cm deep (Figure 3.3). In order to prevent air leakages when the CO₂ flux measurements were made, each shallow collar was sealed on the soil surface using putty (Plumbers Mait, Evo-Stik, Bostik Ltd). The deep collars, 15 cm long, were inserted 10 cm into the soil severing the roots. The vegetation within each collar was removed to prevent new addition of C to the soil.

- a. **RMS** – 6 cm high shallow collars, fitted to the ground with putty for measuring the respiration of roots, mycorrhizal fungi and soil decomposers;
- b. **S** – 15 cm deep collars, inserted in the soil, severing the roots and measuring only the activity of decomposers.

Chapter III

During the four years after the plots were established, the legume abundance declined and by 2012 became patchy. This situation compelled the location of the collars to be chosen using a semi-randomised pattern, in order to coincide with the higher legume density necessary to test the hypotheses formulated. Each pair of collars, comprising of a shallow and a deep collar, was located close together, within a 60 cm x 60 cm area (Figure 3.3). The location of each collar pair was generated in ArcGIS using a randomised pattern. Each location was then checked in the field and approved if it coincided with a legume patch in the GLF plots. If no legumes were present at the designated location, a new location was chosen, by walking over to the nearest legume establishment visible in the field. Once confirmed, the exact location of each collar pair sub-plot was recorded using a Garmin GPS.

Due to unusually wet weather throughout most of the summer 2012, the cutting of the plots was delayed and thus the setup of the experiment in the field. This also resulted in the field having to be returned earlier to the farmer, because of an urgent need for extra land to graze his cattle. In consequence, the length of the experiment was only eight months, from August 2012 to March 2013, not a full growing season, with implications which will be further assessed in the discussion (Section 3.7).

All 48 collars were setup at the end of August 2012, followed by a first set of measurements. The second/last set of measurements was recorded in March 2013 before the experiment had to be terminated. Data recorded consisted of air and soil temperature and moisture measurements and soil CO₂ fluxes.

The equipment used for data collection was the same as the one used in Chapter II (Section 2.5.2). The soil C fluxes were obtained using a portable infrared gas analyser - IRGA (EGM-4; PP Systems, Amesbury, U.S.A.), connected to a 16 cm diameter Perspex chamber (CPY-2; PP Systems, Amesbury, U.S.A.). Three repeated measurements were taken for each collar by placing the chamber on top of the collar rim for 90 seconds, and allowing ambient air to be flushed in, between replicates.

North Wyke collar design

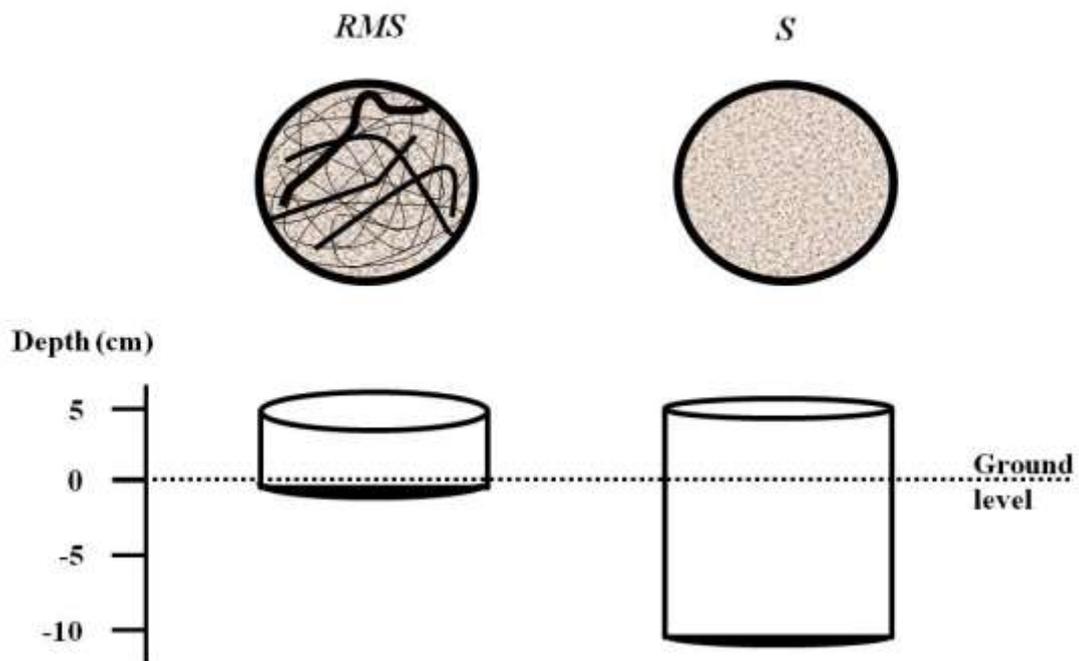


Figure 3.3. Set of collar types and insertion depths. **RMS** - shallow collars measuring all soil respiration flux components: roots (thick lines), mycorrhizal hyphae (thin lines) and soil heterotrophs (grainy pattern); **S** - deep collars excluding both roots and mycorrhizal hyphal in-growth (modified from Heinemeyer *et al.* (2007).

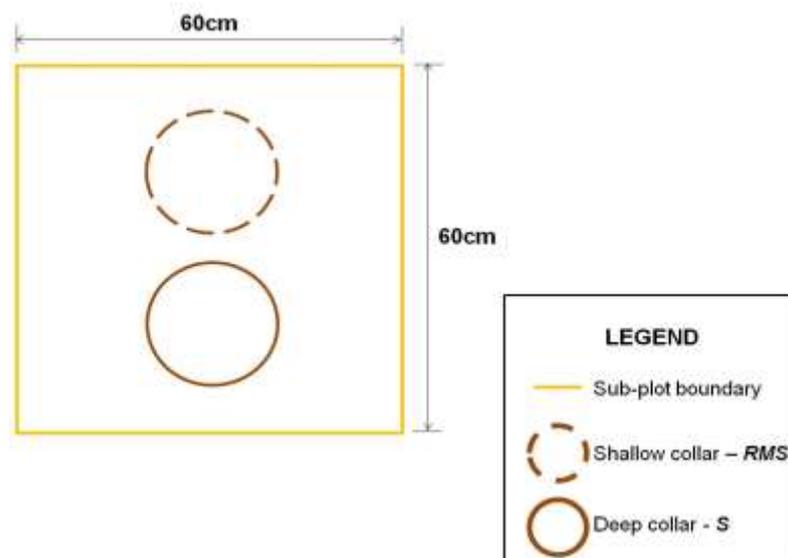


Figure 3.4. Sub-plot containing the two types of collars

Chapter III

3.5.2.3. Litter bags

Wood and grass litter bags were deployed in the field, in order to estimate decomposition rates in the presence and absence of legumes, and later biomass loss was calculated. The grass litter consisted of dried sward cut from the actual plots, while the wood litter was made up of dried tongue depressors. The initial design suggested five time points at which one of each litter bag would be collected, dried and weighed, but due to the early termination of the experiment the last two sets were collected as one. Also, similar to the collar design, the litter bags were added to each plot in triplicate with this within-plot replication (mass loss rates were averaged by plot prior to statistical analysis) being deployed in the same semi-randomised pattern as the collars. Thus, two types of litter x five collection time points x three sub-plots x eight plots, equal a total of 240 litter bags deployed in the field, 120 wood and 120 grass. The bags were retrieved in September and November 2012 and in January and March 2013.

In order to ensure an easy retrieval of the bags at their designated time point, in each sub-plot they were tied up with a wire and then connected to centre point (Figure 3.5) which also corresponded to a set of GPS coordinates. All the litter bags were inserted in the soil at their designated location at approximately 6 cm depth in a 45° angle.

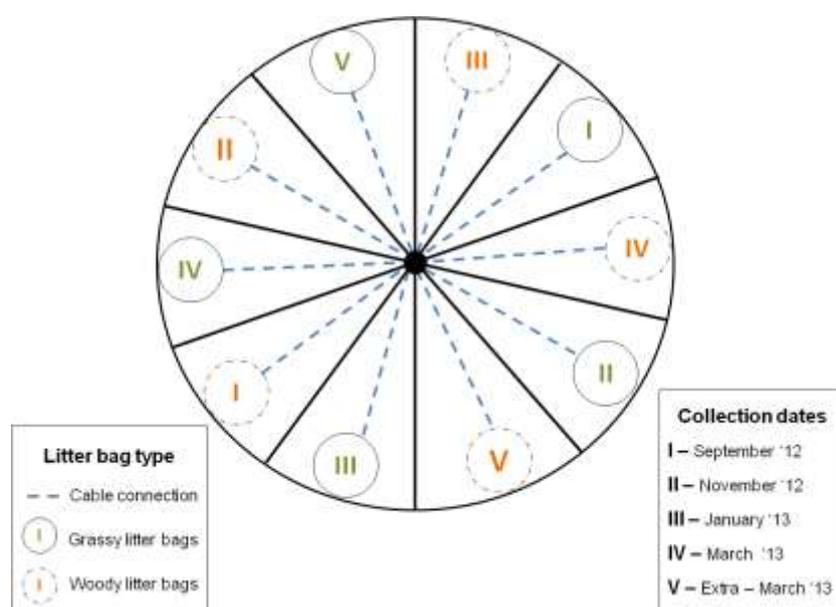


Figure 3.5. Sub-plot layout, including each of the litter bags and the due collection time point

3.5.2.4. *Soil cores*

At the same time each set of litter bags was collected, a soil core was sampled from each subplot. In the end a total of 96 cores were removed from the field, each measuring approximately 30 cm depth and 3 cm diameter. Due to time constraints these were not analysed, but in exchange the team at North Wyke made available data from the monitoring system for the WEB experiments, described below.

3.5.2.5. *Biophysical measurements made by NW team*

The NW strategy comprised of soil core sampling once a year, plus water leachate sampling and vegetation yield measurements three times every year. The soil core sampling carried out by the North Wyke team in 2012 took place during the same period as the experiment described in this chapter, thus making the data obtained relevant.

a. Soil nutrient losses

The soil nutrient losses were determined by collecting leachate samples from each plot, and measuring the total oxidised N (TON) and P concentrations leached from the soil. This was achieved by using two Teflon sampling tubes inserted in soil in Phase 1, at random locations within each plot, to a depth of 60cm. The leachate samples were collected on three occasions (November, January and March) during the winter, and analysed using North Wyke standard laboratory methods STM 246-05 and STM 247-01 (Method B4-1 & Method B4-2, Appendix B).

b. Soil chemistry

The soil sampling was comprised of five cores, 7.5 cm deep and bulked from each plot, in autumn/winter, at the start and end of WEB phase 1 (2008 - 2010) and before the end of WEB phase 2 (2011 - March 2013), during the life of the experiment setup for this chapter. The bulk soil samples were analysed for soil total C and N, TON, Olsen extractable P, total phosphorus (TP) and pH analyses, using standard methods (Allen *et al.*, 1974), described in full in Appendix B (Method B4-2, Method B4-3, Method B4-4 and Method B4-5).

Chapter III**c. Soil structure**

A total of 25 soil penetrometer measurements were made from the surface down to 49 cm below the soil surface within the 500 m² central area of each of the plots under typical management in 2009, 2010 and 2012. This provided a comparative indication of any change in soil surface structure between treatments during the course of the experiment. It was noted that the degree of resistance shown by soils when tested with the penetrometer could be affected by soil moisture. As penetrometer readings were taken over a short period of time (typically within one to two days within a year), under constant weather conditions, differences in soil moisture were not expected to affect between treatment trends. However, between year differences could be confounded to some extent by differences in soil moisture and their interpretation will be considered.

In addition, dry soil bulk density determinations were made. Ten soil cores of 4cm diameter and 20cm deep were taken from each typical treatment plot. Each core was sub-divided into a surface 0-10 cm depth section and a 10 – 20 cm depth section. Each section was weighed fresh and then crumbled and dried to constant weight. Any stones of more than 6 mm diameter and any large roots (e.g. tap roots) were removed from a soil section and their total volume measured by volume increase of a known volume of water. The soil bulk density measurements at the two depths provide an indication of whether the establishment of deep rooted species can ameliorate soil structure in the lower soil horizons.

d. Vegetation structure

Another measurement made as part of the monitoring programme at NW was the herbage yield from June, August and September of each year and species mean cover calculated yearly. To assess the yield of herbage produced during a silage cut swaths left after the tractor cut were weighed and sampled from a known area to provide a measure of dry matter yield. Samples were also taken to assess forage quality (mineral content and digestibility). Mineral (N, P, Ca, Mg and Na) and pepsin cellulose digestibility analyses were undertaken for these samples. Determination of total N was by use of the Leco FP 428 N determinator. Determination of extractable P in herbage was by colorimetry, and that of Na, K, Ca and Mg by ICP-AES. All of this data was made available and the most relevant ones were processed and presented in the results section.

3.5.3. Calculations and statistical analysis

The data collected during this experiment was recorded and corrected in Microsoft Excel (Microsoft Office Professional Plus 2010, ©2010 Microsoft Corporation). For illustration of the data sets, graphs and figures were created in Microsoft Excel. The data was encoded and analysed for statistical significance using SPSS (IBM® SPSS® Statistics 20). Initial analysis determined that all the data sets were normally distributed, followed by Mauchly's test of sphericity and Levene's test of equality. In order to test whether there was a significant difference between the two types of treatments (G and GLF) within the typically ploughed managed plots, the litter decomposition and soil CO₂ flux data was examined using independent *t*-tests. Independent *t*-tests were also used to test the difference between the two collar treatments in each treatment plot for both sampling sessions. Two-way ANOVA was employed to investigate the effect of treatments and collars on soil CO₂ fluxes, while the interaction effect between treatments, collars and blocks was tested by three-way ANOVA. The change with time of all these factors and the interactions between them was tested using repeated measurements ANOVA.

Similar to the data collected in the calcareous grassland in Peak District, it was possible to calculate the autotrophic and heterotrophic components of the soil CO₂ fluxes. The respiration rate measured at the *RMS* collars was considered to represent total below-ground respiration from all components, and *S* represents the soil decomposers component. Thus the contributions of roots, mycorrhizal fungi and soil decomposers respiration were calculated as follows:

[Eq. 12] Heterotrophic respiration (**soil decomposers**) = **S**

[Eq. 13] Autotrophic respiration (**roots + mycorrhizal fungi**) = **RMS – S**

In order to assess the percentages of each respiration component the following equations were used:

[Eq. 14] **(Root + mycorrhizal fungi) % = ((RMS – S) / RMS) * 100**

[Eq. 15] **Soil decomposers % = (S / RMS) * 100**

3.6. RESULTS

3.6.1. Soil temperature and moisture

Results indicate that the soil temperature decreased, while the moisture increased with depth, both in September 2012 and March 2013. There was a significant difference in both soil temperature and moisture with depth ($p < 0.001$) and from one sampling session to another ($p < 0.001$). Also, the soil temperature was significantly different between the two collar types in March 2013, while moisture was similar on both occasions (Table B2-1, Appendix B). The treatments applied showed no significant effect on soil temperature, but there was a marginally significant difference between G and GLF for soil moisture ($p < 0.08$).

The soil temperature and moisture data showed a significant correlation ($r^2 > 0.9$), but the points were clustered into their respective sampling session and depth, indicating not a spatial correlation but more likely a change with time of day and season (Figure B2-1, Appendix B).

3.6.2. Soil CO₂ fluxes

The initial repeated-measurements ANOVA showed significant main effects of collar ($p < 0.001$), block ($p = 0.010$) and time ($p < 0.001$), as well as some interactions between these variables (treatment ↔ block; treatment ↔ collar; time ↔ collar). In September 2012, a couple of weeks after installation, respiration rates were greater at the end of the experiment in March 2013 (Figure 3.6), when the temperatures were much lower. Possibly related to disturbance caused by collar insertion, there was a marginal higher rate of respiration from the deep collars in September 2012, but this difference was lost by March 2013.

The p values, obtained after analysing the soil temperature and moisture and soil CO₂ fluxes, are summarised in Table 3.1. These represent the significant differences recorded between treatments and other measured parameters like

collar type, or sampling depth, as well as the interaction between them on both sampling sessions. The statistically different p values are reported with an (*).

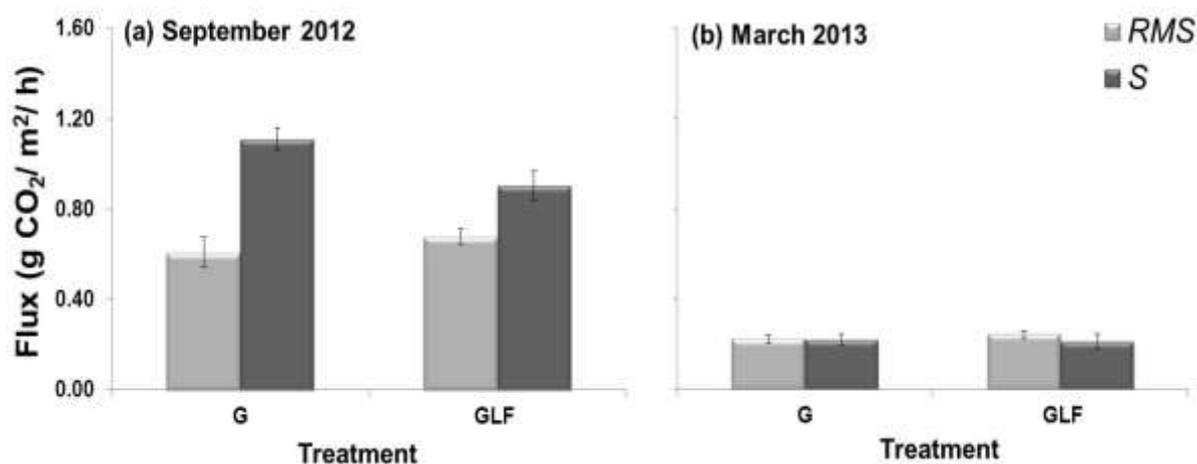


Figure 3.6. Soil CO₂ fluxes recorded in September 2012 (a) and March 2013 (b) for both treatments (G & GLF) at the RMS and S collars. Error bars are ± 1SE (n=3)

Table 3.1. Summary of p values obtained for temperature, moisture and flux by 1-way ANOVA statistical analysis, in September 2012 and March 2013; values with (*) are statistically different

			TEMPERATURE	MOISTURE	FLUX
TIME	SEPTEMBER 2012	Treatment	0.657	0.145	0.422
		Collar	0.422	0.604	0.000*
		Depth	0.001*	0.000*	-
	MARCH 2013	Treatment	0.218	0.353	0.403
		Collar	0.027*	0.847	0.000*
		Depth	0.002*	0.000*	-

3.6.3. Heterotrophic and autotrophic respiration

The partitioned soil respiration obtained from the two types of collars installed, indicate that the main contributor to the soil respiration rate was the heterotrophic component while the autotrophic one was significantly lower (Figure B3-2). The autotrophic respiration calculated for September 2012 revealed negative values, which indicated that for September 2012, measurements of the method used to measure the soil respiration components failed. This may have been caused by increased fluxes due to the disturbance caused by collar insertion, or possibly by windy conditions creating negative pressure in the chamber or water logged soils, displacing the CO₂ from the soil profile.

3.6.4. *In-situ* litter decomposition

Figure 3.7 illustrates the difference in mass loss observed between the two litter types, indicating that they decomposed at different rates during the 32 weeks that they were left in the field. The more readily decomposable material of grass litter was lost in a much higher percentage than the recalcitrant one from the woody bags. Decomposition rates were expected to differ between the G and GLF plots as a result of the legume influence on C and N availability and demand. After only six weeks the grass litter from both plot types lost over 65% of their mass without any difference between treatments, reaching a total of approximately 85% loss by week 32 at the end of the experiment (Figure 3.7 – a). However, by week 24 there was a marginally higher mass loss ($p < 0.08$) in the GLF compared to G plots. This difference was completely lost by week 32.

The wood litter mass loss is presented in panel (b) of Figure 3.7. The mass loss of wood litter was between 3% – 8% in each treatment plot. There was a trend towards greater mass loss in the GLF plots than in G plots. While there was no significant difference between treatments on the first and second retrieval session, on the third sampling session, calculations revealed a marginally significant difference ($p = 0.075$), and significant difference ($p = 0.035$) on the last session.

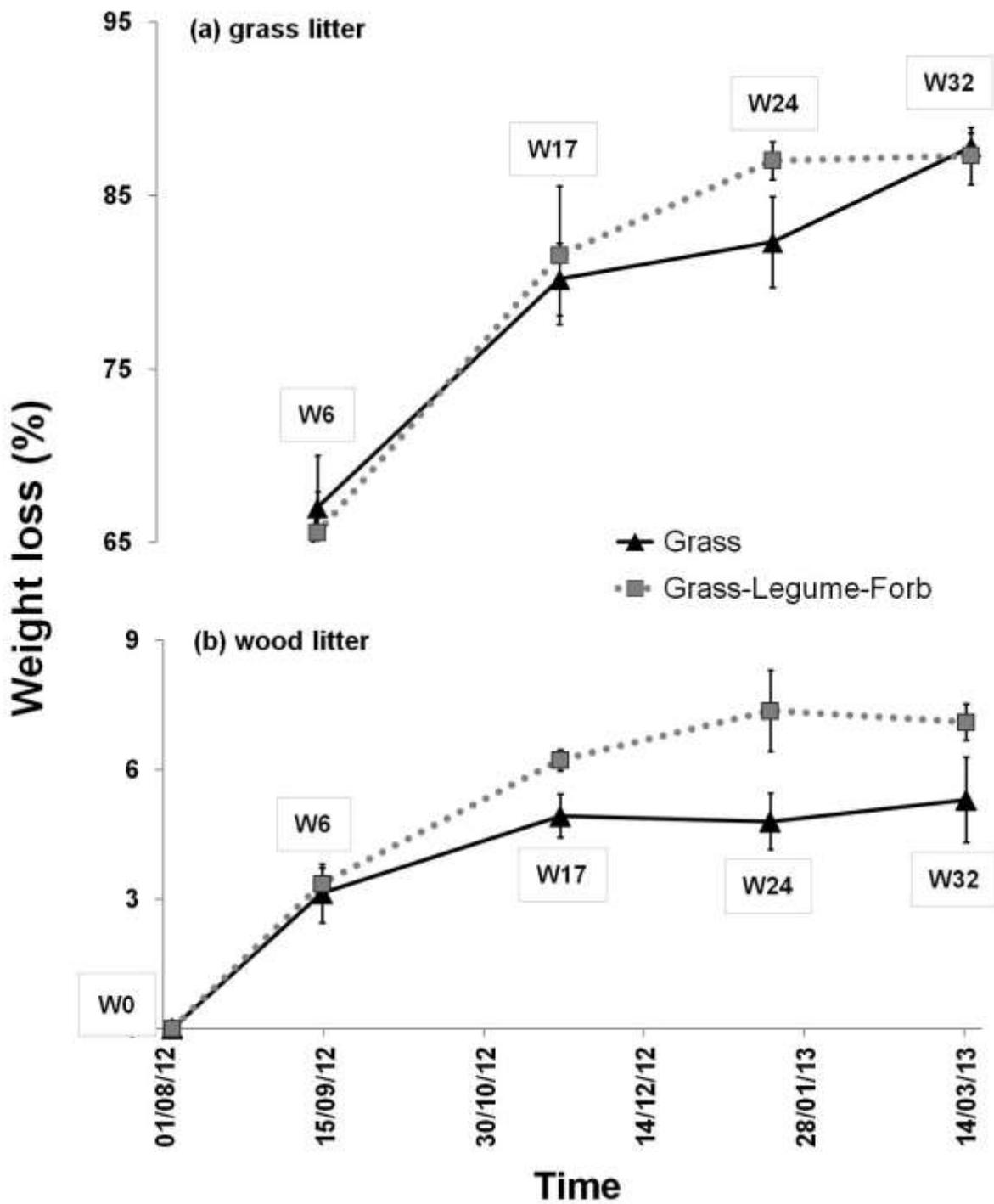


Figure 3.7. Mean weight loss (%) for the two types of litter (a – grass, b – wood), collected at four time points during the growing season (September, November, January and March)

3.6.5. Soil physical and chemical characteristics

The data presented in this section were made available by the team at NW and were analysed independently from them, using the same methodology as the rest of the data collected specifically for the experiment described in this chapter. The data provided by the team at NW, was collected between 2008 and 2012. Where a year is not mentioned, it is due to a lack of measurements during that year.

3.6.5.1. *Moisture, compaction and bulk density*

Measurements of soil moisture revealed that even though there was no significant difference between the values recorded at 0-10 cm and 10-20 cm depth in either of the treatment plots, there was a marginal difference between the years that they were measured in: 2009, 2010 and 2012. Year 2010 was the driest of the three, with mean soil moisture under 25 %, while 2012 was the wettest, with values over 30 %. The soil bulk density measured at the same depth intervals, 0-10 cm and 10-20 cm, showed no significant difference between each other or between treatments (Figure B2-2, B3-3, and B2-4, in Appendix B).

3.6.5.2. *Soil C, N, P and pH*

The parameters were analysed for soil samples taken in 2008, 2011 and 2012 only. Yearly values of total soil C, N and phosphorus, showed no significant differences between G and GLF treatments or sampling sessions, but they were significantly higher in 2008 and 2011 for the control plots where the original grassland remained undisturbed (Figure 3.8 – a, b, c).

Marginal differences were recorded for the soil Olsen P, where the values in 2008 were higher than in 2011 and 2012, with the lowest concentration in 2011 (Figure 3.8 – d). In 2008 and 2012, values were marginally higher in the G plots compared to GLF and significantly higher for the original grassland plots in 2008 at the beginning of the experiment.

Chapter III

The soil pH remained approximately unchanged during the entire duration of the experiment (Figure 3.8 – e) and showed no difference between treatments or sampling sessions.

The C/N ratio indicates the requirements of the soil in order to optimise the demand and consumption of the two elements. In this case, a higher ratio will result in slower decomposition rates (Table 3.2). No significant difference was observed between the ratios in the two treatment plots.

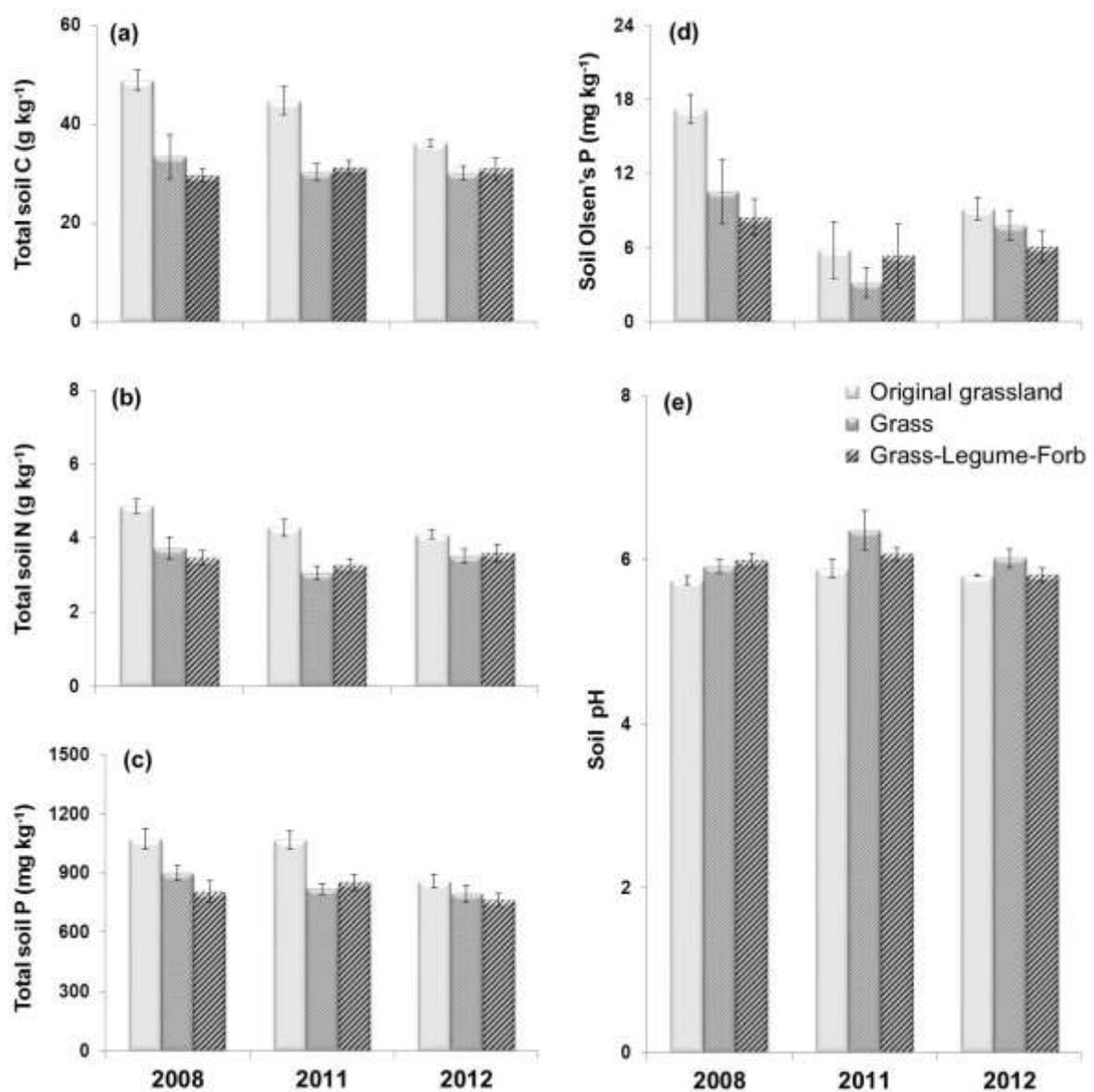


Figure 3.8. Mean soil C, N, P and pH (\pm SE) in the first year of the experiment 2008, and the last two; 2011 and 2012

Table 3.2. Summary of the C/N ratios calculated in the G and GLF plots, in 2008, 2011 and 2012

		C/N	±SE
2008	G	8.9	0.40
	GLF	8.5	0.09
2011	G	9.9	0.31
	GLF	9.6	0.16
2012	G	8.6	0.14
	GLF	8.6	0.08

3.6.6. Vegetation cover, diversity and yield

Analyses of the sward cover for every year of the experiment since 2008 is presented in Figure 3.9. Legume mean cover shows a big decrease in the GLF plots from over 60 % in 2009 to less than 5 % at the end of the experiment; with over 30 % loss after the first year and approximately 10 % every year after. At the same time the legumes started to increase in the G only plots, where the legume cover increased from 0 % in the first two years after sawing to 0.2 % in 2011 and 2.8 % in 2012 (Figure 3.9 – a). Forbages were only present in the GLF plots and they too suffered a decrease in cover from approximately 40 % in 2009 and 2010 to under 10 % at the end of the experiment (Figure 3.9 – b), reaching maximum cover in 2010 with over 44 %.

The main sward in all the plots was grass which had a constant higher mean cover in the G plots than GLF, especially in the first two years when they were established. In the last years before completion, the difference became smaller (Figure 3.9 – c). The vegetation yield is illustrated in Figure 3.10 and summarised in Table 3.3., showing consistently higher values for the GLF plots, though significant only in the first two years after establishment, with only marginally significant differences in the last two years.

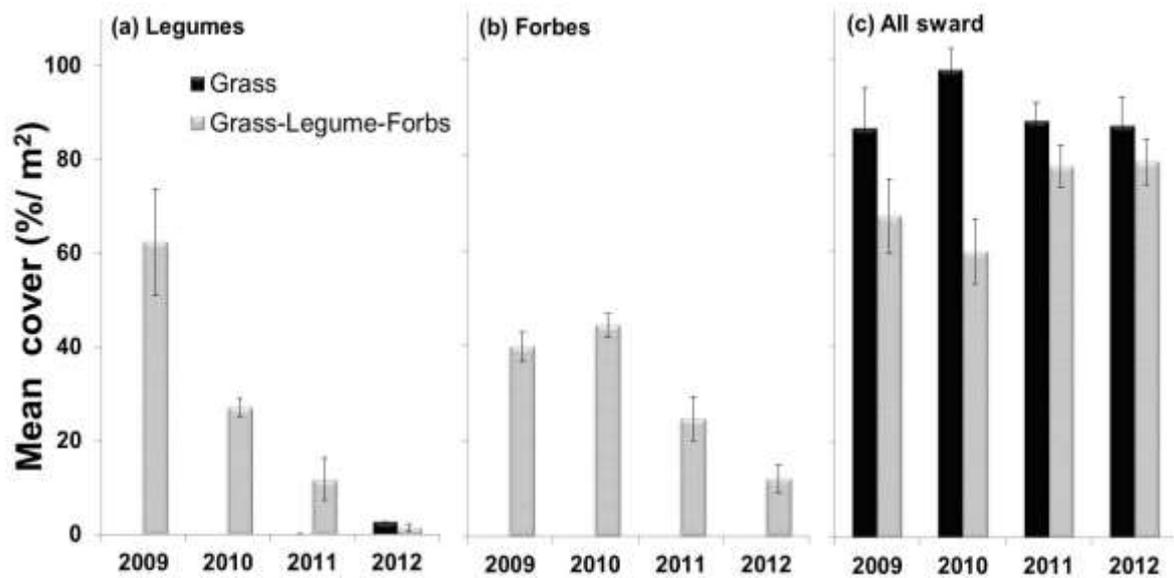


Figure 3.9. Mean legume, forb and all grass cover (% m⁻²) for each year of the experiment (2009 to 2012) in the G and GLF treatment plots

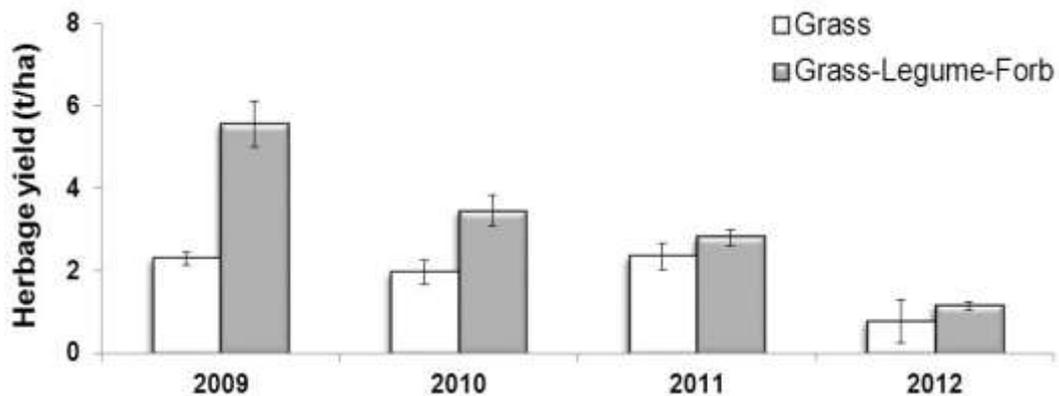


Figure 3.10. Mean yearly herbage yield calculated for the typical cut plots under grass and grass-legume-forb sward. Error bars are \pm SE.

Table 3.3. Typical cut plots (G and GLF) herbage yield, reported as tonnes per ha and the calculated standard error, for all four years measured (2009 to 2012). Differences in yield between the two seed mixtures (GLF-G) and percentage GLF yield is higher than G yield.

	G	\pm SE	GLF	\pm SE	GLF - G	% (GLF>G)
2009	2.29	0.57	5.55	1.08	3.26	58.7
2010	1.95	0.38	3.44	0.61	1.49	43.3
2011	2.33	0.19	2.81	0.55	0.48	17.1
2012	0.77	0.10	1.14	0.12	0.37	32.5

3.6.7. Soil nutrient loss

The nutrients lost by leaching into the soil solution were analysed and results presented in this section. The data recorded showed high variability on all sampling occasions for all treatments, thus no statistically significant difference was observed. In order to reduce some of this variability, values collected three times a year in January, March and November were averaged as yearly concentration levels (Figure 3.11).

Overall, results indicate that seed mix did not significantly affect total P or TON concentrations. However, between 2009 and 2012 TON levels were 62.8 % higher in the GLF plots compared with the G plots, and 55.8 % higher than those recorded for the same period in the original grassland plots. Also, in 2012, TON was 50 % higher in GLF than G with values consistently higher in all blocks.

Leachate analysis for total P measured from 2009 to 2012 indicates marginally higher values for the GLF plots. Though very variable, the data recorded during this period in the GLF plots show a 49.3 % higher P concentration compared to G plots but only 6.4 % higher compared to the original grassland plots.

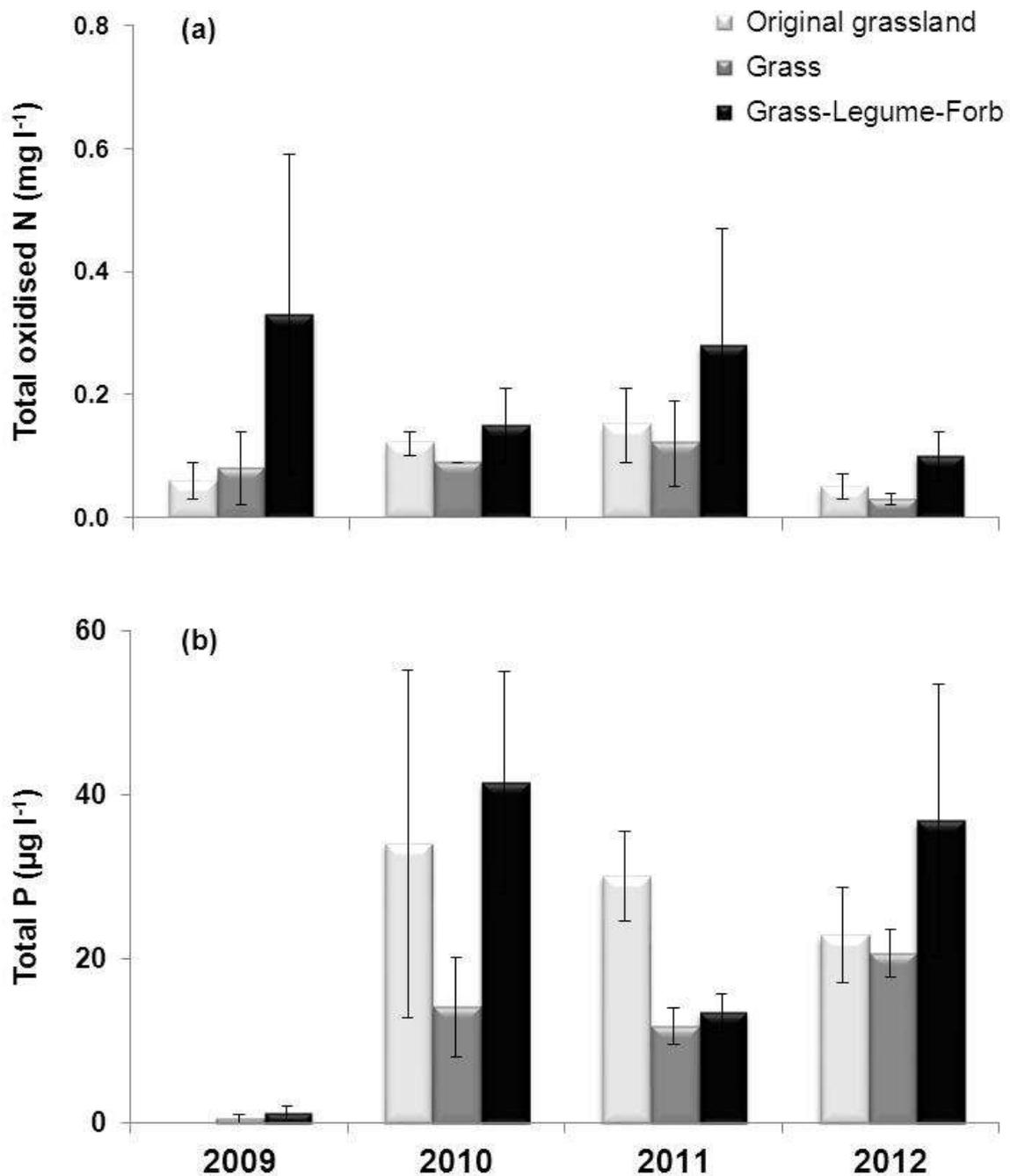


Figure 3.11. Mean total oxidized N (a) and mean total P (b), measured in water leachate collected from 2009 to 2012, for the original grassland plots, grass plots (G) and grass-legume-forb plots (GLF). Error bars are \pm SE (n=4).

3.7. DISCUSSION

Although BNF was not measured directly during the course of these experiments, nor as part of the monitoring schedule at North Wyke Research Centre (NW), the information gathered represents indirect evidence that some N₂ fixation took place. Agricultural management employed by the NW team, and field conditions during the 2008-2013 period, enabled legume growth and development, thus possibly N₂ fixation too. Even though the legume abundance was at an all-time low in 2012, when the additional experiment was setup for this chapter, there are reasons to assume that the previously fixed N was still present in some proportion in the soil pool.

Overall, the main aims and objectives of this research were met. Differences in productivity, soil respiration rates, soil nutrient availability and litter decomposition rates in the presence/absence of legumes was assessed. Neither of the two hypotheses formulated initially were supported. After the initial perturbation caused to the soil by inserting the collars, heterotrophic and autotrophic below-ground respiration did not differ significantly between the G and GLF plots. Furthermore, contrary to expectations decomposition rates of woody litter was increased in the presence of legumes.

3.7.1. Changes in soil physical and chemical characteristics

A small difference was observed between the soil bulk density in 2009 and the following two years when the measurements were taken. The first year after the soil was initially ploughed in order to apply the treatments, the soil loosened, and became less dense, but after each year, precipitation and mild human traffic made the soil denser. Thus, the soil compaction is related to the activities that took place during the five years since the sward seeds were first sown in the field.

Analyses of soil C, N and P stocks, showed no significant differences between the two treatments, thus the influence of legume presence is not easily determined. The only observation to be made here is that, in the cultivated plots the soil C, N and P declined compared to the original grassland, possibly due to the change in

Chapter III

management and increased human activity. There is, however, a slightly higher concentration of these elements in the GLF plots in 2011 and 2012, though not significant. The magnitude levels recorded, might be masked by factors unknown, but this could represent some evidence for the positive effect of legumes on the soil properties.

The presence of legumes is known to decrease soil pH; well nodulated legumes take up more cations than anions, thus resulting in decreases in rhizosphere pH and eventually in bulk soil pH (Tang *et al.*, 1999). The results show that once legumes were established, there was a decline in soil pH in the GLF compared to the G plots, though not significant. This could be explained by the presence of legumes and potentially N₂ fixation.

Perhaps the most important observation was that total oxidised N concentrations, were much higher in the GLF plots than the G plots especially early in the experiment, although these difference were not always statistically significant. Even though total soil P showed no significant difference between the plots with and without legumes, the total P leached into the soil solution showed constantly higher values in the GLF plots compared to the G plots, regardless of the high variability of the data. This could be an indication of excess P leaching into solution, while supporting the evidence of N₂ fixation which was greater when sufficient P was available. Studies support this fact, confirming almost double N fixation estimates when P additions were made to the soil (Binkley *et al.*, 2003, Isaac *et al.*, 2011, Sorensen *et al.*, 2012).

3.7.2. Ecosystem productivity, decomposition and CO₂ efflux

In the plots where legumes were present the herbage yields were consistently higher than those with grasses only, four years in a row (2008 - 2011). The difference in yields between the plots with and those without legumes decreased as the years passed, from 3.26 t ha⁻¹ in 2009 to 0.37 t ha⁻¹ in 2012, due to the reduction in legume and forb coverage. At the same time, the percentage by which the GLF plots were more productive than G, decreased from 58.7 % to 17.1 %.

It is known that N₂ fixation by pasture legumes is regulated by a natural feedback mechanism that is mediated through changes in soil inorganic N and competition from associated grasses (Ledgard *et al.*, 1998). The greater total oxidised N in the GLF plots, could partially explain why the coverage of the legumes in the GLF plots gradually declined over time. In addition, legumes may have often short growing seasons and may be subject to intermittent or terminal drought, and can be outcompeted for resources including light (Gundale *et al.*, 2012). The limited legume productivity can also be caused by acid soil conditions and gradual degradation of soil chemical and physical properties. Lockwood *et al.*, (2003) suggested that the decrease in soil pH is typically linked to N leaching from legume root zone.

Decomposition rates, on the other hand, showed a different trend. It was hypothesised that rates would be lower under cultivated legumes, but the wood litter decomposition rates were actually greater in the GLF plots. This could have been related to the higher sward biodiversity recorded in the GLF plots, where plant species richness can influence decomposition by impacting the quality of the litter and the microclimate in which the litter decomposes. It is possible that the soil microbial community present in the more diverse GLF was better able to break down a novel litter type, with the wood being more similar to substrates produced by some of the forbs (Keiser *et al.* 2013 and 2011). The high lignin content in the wood litter could be responsible for the observed behaviour. Even though lignin is typically considered a recalcitrant material resistant to microbial decomposition, it can be broken down to usable forms, by specialised biota like fungi (Austin and Ballaré, 2010). Increased plant biodiversity due to the presence of legumes, and forbs, can increase associated decomposers and possibly even those able to increase wood litter decomposition, explaining the observed trend.

In general, higher N concentrations have been found to inhibit the breakdown of high lignin-content litters (Knorr *et al.* 2005), and thus it seems unlikely that a reducing N limitation in the soils could have resulted in a microbial community that was better able to decompose the high C-to-N ratio woody litter. It was not possible to determine if there was any inhibitory legume cover effect earlier in the

Chapter III

experiment, when legume cover and soil N concentrations were greater. Further study is required to identify the mechanisms involved, but whatever the explanation, it is clear that the hypothesis of reduced decomposition under legumes was not supported by this study.

Soil CO₂ flux measurements were limited to two sampling sessions due to weather conditions. The lack of sufficient sampling sessions throughout the growing season limits the conclusions which can be reached. The initial set of measurements were taken not long after collar insertion, thus, part of the magnitude of the recorded values can be explained by disturbance to the soil. On the second sampling session, the atmospheric temperatures were very low, and so were the measured soil CO₂ fluxes, making it difficult to detect a significant difference between treatments.

3.8. CONCLUSIONS

Evidence gathered throughout this study supports previous research, confirming the beneficial influence of legumes planted in grasslands for the purpose of increased soil fertility, and ecosystem productivity. Contrary to expectations, decomposition rates increased in the presence of legumes, although the mechanism underlying this response could not be determined. Overall the presence of legumes lead to increased plant diversity, higher greater plant biomass and productivity, and thus soil C and N inputs, but also to greater decomposition rates. Therefore the net effects on soil C storage require further study.

CHAPTER IV

THE IMPACT OF NUTRIENT ADDITIONS, WARMING AND CLIPPING ON ECOSYSTEM PRODUCTIVITY AND CARBON STORAGE

4.1. INTRODUCTION

4.1.1. Research questions

The constant changes in our environment due to human alterations have affected both the nitrogen and carbon cycles. Evidence gathered in Chapters II and III suggests that nutrient addition to the terrestrial ecosystem, from either organic or inorganic sources, can have a positive effect on the soil fertility, ecosystem productivity and potentially carbon storage. In addition to this, temperature also plays a major role in controlling the rate of C and N cycling in terrestrial ecosystems. These individual factors have been shown to have different effects on the soil respiration rates. However, their interactions could be the key to understanding the future of carbon release from soils and thus the potential to mitigate climate change.

A predicted global average temperature increase of 1.1 – 6.4°C during this century (IPCC, 2013), has the potential to affect terrestrial ecosystem processes, such as soil carbon dynamics (Davidson and Janssens, 2006, Cheng *et al.*, 2011). Almost 80% of the terrestrial C pool is stored as soil organic matter (SOM) and is considered an important potential C sink that may

help offset the greenhouse effect (Lal, 2008; Maia *et al.*, 2010). The degree to which increasing temperatures cause decomposition to deplete SOM stores and provide a positive feedback to global warming is still a major uncertainty in the ability to predict future CO₂ levels (Thiessen *et al.*, 2013). In addition, warming-induced changes in SOM decomposition regulate the availability of nutrients for plant growth and ultimately influence the net primary productivity of terrestrial ecosystems (Cheng *et al.*, 2011). Hence, it is imperative to understand how global warming will affect these soil process dynamics. The response of soils to warming depends on many factors, such as soil moisture and carbon inputs to soils (Cheng *et al.*, 2011), but may also depend on the nutrient availability to the ecosystem.

An improved understanding of how temperature and nutrient availability interact to control ecosystem carbon storage is critical for predicting future rates of climate change and for decision-making aimed at mitigating climate warming.

4.1.2. Current perspective

Under climate change, the terrestrial carbon balance factors other than the direct effects of temperature on decomposition will be important in determining changes in ecosystem C storage. Reductions in soil moisture and increased plant respiration associated with warming will tend to reduce carbon storage in a variety of ecosystems at all latitudes. On the other hand, increased nutrient availability could reduce the microbial activity and increase carbon storage. The possibility that global change can trigger both positive and negative feedbacks to the climate system (Melillo *et al.*, 2002), highlights the importance of an accurate representation of all these interactions in order to predict with more accuracy the climate change over the next decades.

4.1.2.1. *The need for linking plant and soil measurements*

The initial C accumulation in soil, especially in the northern hemisphere, is considered to have been promoted by cold and wet conditions that inhibit decomposition of dead plant tissue that enters the soil organic matter pool (McGuire *et al.*, 2009). Contrary to that scenario, the present atmospheric warming trend could encourage the reversal of this process and the release of vast quantities of C to the atmosphere, further accentuating global warming. Thus, growing interest in the fate of soil carbon storage in a warmer world has triggered research investigating some consequences under different scenarios. One of the key factors that might affect the potential for C release under warming is nutrient availability. Not enough has been done to help understand the impact of the interactions between temperature and nutrient availability on ecosystem C storage.

Investigating the effects of warming on soil C dynamics requires a relatively stable and easy to control environment. This encouraged a large number of investigation into the fate of soil C stocks, by incubating bulk soil samples (Conant *et al.*, 2008a) or fractions representing different SOM pools (Plante *et al.*, 2010, Stewart *et al.*, 2009) and measuring the change in CO₂ fluxes over time. However, there are substantial experimental artefacts in these studies: C inputs from plants are lost resulting in C depletion and changes in microbial community structure (Steinweg *et al.*, 2008). Furthermore, to determine how the ecosystem C balance is affected it is extremely important investigate if the potential increase in nutrient availability caused by greater rates of decomposition can promote plant growth (Melillo *et al.*, 2002). Therefore it is necessary to carry out *in-situ* warming experiments using intact plant-soil systems.

4.1.2.2. *The role of C₃ and C₄ in carbon cycle research*

In the late 1960s a very important discovery was made; terrestrial plants show a clear distinction in $\delta^{13}\text{C}$ values (Bender, 1968). Trees, shrubs and many grasses are C₃ plants, with lower $\delta^{13}\text{C}$ values that average near -28 ‰, whereas the C₄ plants like corn, sugar cane and dry-land grasses, have higher values reaching almost -13 ‰. These isotopic differences appear as a result of photosynthesis when carbon atoms are incorporated into 3-carbon (C₃) or 4-carbon (C₄) sugars, by formation of a new chemical bond (Fry, 2006).

The C isotope ratio of SOM is close to that of the source plant material; as a result, soil organic matter is richer in ¹³C (i.e., the $\delta^{13}\text{C}$ ratio is less negative) when the plant material is derived from C₄ rather than C₃ plants. Thus, introduction of C₃-derived organic matter into a soil that was previously colonized by C₄ vegetation, represents an *in-situ* labelling of new organic matter incorporated into the soil (Martin *et al.*, 1992). Therefore, analysis of the ¹³C gives the opportunity to trace any new inputs of carbon in a system that has had a history of different C source inputs and follow its behaviour under different climate conditions.

Using the carbon isotopes to trace the large exchange between atmospheric CO₂ and the soil C storage can help explain the interdependence and potential feedbacks to climate change. Thus, using a C₄ soil and a C₃ plant in the experimental design, it is hoped that different sources of CO₂ released from an ecosystem can be identified, and changes in the stocks of old versus new SOM can be measured. Improving the representation of soil C dynamics in models by looking at SOM formation versus release (Chapin *et al.*, 2009), has important implications for understanding the patterns and rates of climatic change.

Previous studies suggest that C losses from a terrestrial ecosystem can be tracked using the $\delta^{13}\text{C}$ values by linking the mobilised and transported material in order to understand movement of material around environments (Puttock *et al.*, 2012). This material can be either soil eroded and transported by surface

waters, dissolved organic matter, litter inputs into the soil, but most importantly SOM of different ages. Also, it was found that the C fraction in litter can be twice as much as the fraction released as CO₂ to the atmosphere (Dungait *et al.*, 2011, Rubino *et al.*, 2010), making the study of isotopic signatures of different C highly relevant and significant.

4.1.3. Summary

It is widely recognized that C sequestration in terrestrial ecosystems can be critically influenced by the SOM decomposition response to temperature (Hartley and Ineson, 2008, Jenkinson *et al.*, 1990, Kirschbaum, 1995). Root activity (Hanson *et al.*, 2000) in the field and rapid changes in the dynamic labile SOM pool (Gu *et al.*, 2004) in the laboratory, are just a few interferences affecting accurate C efflux measurements. Some incubation results suggest that the temperature sensitivity of SOM decomposition increases with substrate recalcitrance (Hartley and Ineson, 2008). This is supported by knowledge gathered so far from research, suggesting that there is increasing potential for positive feedback effect between global warming and soil-carbon stocks (Schimel *et al.*, 1990; Kirschbaum, 2004) and therefore predictions of future C release from soils may be underestimated. As mentioned earlier, there are multiple limitations associated with direct measurements of changes in soil C stocks, both in the laboratory and *in-situ*, thus the focus of research was shifted towards measuring the soil C fluxes (Valentini *et al.*, 2000).

Chapin *et al.*, 2009, identifies a few critical gaps in the modelling of ecosystem-climate feedbacks and suggests further research into nutrient and other controls that couple photosynthetic C input to respiratory C outputs. In addition, there is a need for better understanding of the connection between the increase in temperature caused by an increase in atmospheric CO₂ and the processes governing the net C balance of the terrestrial biosphere.

4.2. WORK AND SITE JUSTIFICATION

The work necessary to meet the aims and objectives of this chapter was carried out in laboratory conditions in the Experimental Hall of the Geography Department at the University of Exeter. Controlled conditions were necessary due to the handling of foreign soil used for this experiment. Also, the manipulation of soil temperature, added fertiliser and clipping, plus monitoring of the added and leached water needed to test the hypotheses, was only possible in a controlled environment.

Given the research requirements identified above, it was important to design an experiment that could manipulate nutrient availability, temperature and other environmental factors. At the same time having the facilities to make frequent day and night flux measurements, control the water added and collect the leachate for testing, made the experimental area at the University the perfect place to setup and run this experiment.

4.3. AIMS AND OBJECTIVES

The main aim of the work described in this chapter was to determine how nutrient availability and plant soil interactions affect the rates of carbon release from the soils in response to warming. The main objectives were:

O1. To measure CO₂ fluxes in from a replicated factorial soil warming experiment using bare soil and planted lysimeters, receiving high or low additions of nutrients, and differing in plant clipping regimes.

O2. To quantify the release of carbon from C₃ or C₄ sources in the presence/absence of vegetation, due to different nutrient additions and temperature change.

O3. To monitor physical and chemical changes and place these results in the context of key ecosystem and soil properties: grass yield, total soil C, N, P and water soluble N and P.

4.4. HYPOTHESES

To meet these objectives, the following key hypotheses were tested:

H1. Increased nutrient availability will reduce rates of decomposition of old SOM due to a reduction in priming effects.

H2. Warming will tip the ecosystem carbon balance from net uptake to release, but this effect will be reduced when nutrient availability is low, because warming will increase soil nutrient availability promoting plant C uptake which could compensate for soil C losses.

H3. Clipping will stimulate C loss from the planted lysimeters due to less potential for warming induced increases in nutrient availability to promote plant growth.

H4. The carbon stocks in soils will be reduced by warming, but to a greater extent in the high nutrient treatment.

4.5. METHODOLOGY

4.5.1. Site and experimental design

All experiments were carried out indoors under controlled laboratory conditions. The experimental design consisted of 48 custom-made PVC-containers, set up as lysimeters, containing different layers of C₄ soil, sand and gravel, with or without a C₃ grass planted inside. The lysimeters were divided into four replicate blocks, each consisting of 12 full factorial treatments as described in Table 4.1. The entire experiment ran for 66 weeks from the end of April 2012 until July 2013.

Table 4.1. Factorial experimental design, illustrating all 12 treatments (a - l) with three factors: each having two levels (nutrient and warming: 0 – no nutrient/ control; 1 – high nutrient/ warming, unclipped biomass; 1 – clipped biomass).

	Treatment	Nutrient	Warming	Clipping
1 plant – grass	Soil only lysimeters			
2 nutrients – low and high	a	0	0	n/a
2 temperatures – ambient and warmed	b	0	1	n/a
2 clippings – unclipped and clipped	c	1	0	n/a
	d	1	1	n/a
X 4 replicates	Planted lysimeters			
Planted lysimeters:	e	0	0	0
1 x 2 x 2 x 2 =	f	0	0	1
8 treatments x 4 reps = 32	g	0	1	0
Soil only lysimeters:	h	0	1	1
2 x 2 =	i	1	0	0
4 treatments x 4 reps = 16	j	1	0	1
	k	1	1	0
	l	1	1	1
48 LYSIMETERS				

4.5.1.1. *Lysimeters and warming system*

The lysimeters were built using 20 cm diameter PVC pipe cut into 48 collars, straight at the top and at an approximately 45 degree angle at the bottom, enhancing the drainage capability of the system; the shortest side of each collar measured approximately 15 cm and the longest 25 cm. The bottom of each collar was sealed with elliptical PVC sheets, after a 2 mm hole was drilled at one end, to allow the leachate to drain. The warming system was designed to increase the temperature in specific lysimeters in order to test the hypotheses. This was achieved by pumping warm water through meters of 6 mm diameter PVC tubing, from a water tub with a thermostat, leading to the lysimeters and back to the water tub. On two opposite sides of each lysimeter, six holes were drilled (three up and three down), and the tubing was inserted staggered on two rows according to Figure 4.1.

4.5.1.2. *Frame and lights*

Due to the angled bottom of the lysimeters, a support was built to keep them level. This was achieved by linking together 5 cm diameter metal scaffolding pipes, and creating a 2 m by 2 m wide and approximately 3 m high frame. Within this 4 m² area, additional pipes were placed at approximately 20 cm apart to allow the lysimeters to fit, and on a lower level at 6 cm under the main frame, another set of pipes was connected to support the angled bottom of each lysimeter. Under these two levels of pipes supporting the lysimeters, a third level was created adding one more pipe on two opposite sides. This third level was used to support a wood plank and create a shelf under the lysimeters, on which trays with beakers were placed for collecting leachate.

In order to achieve a controlled number of hours under uniform daylight, necessary for plant growth, additional lighting was installed. Four fluorescent 400 W lights were attached to the metal frame in each corner, at approximately 2 m above the lysimeters. These were then connected to timers, providing 12 h of light during the summer and 8 h during the winter. The completed frame and lighting system are illustrated in Picture C1-1 (Appendix C).

4.5.1.3. Soil and seeds

The soil used for this experiment was collected at the Konza Prairie LTER site in Kansas, USA. The organic matter in this soil was primarily derived from C₄ grasses, giving it a strong isotopic signature, allowing new inputs to be traced, making it perfectly fit for the purpose of this experiment and for testing some key hypotheses. Konza Prairie soils have a pH of around 6 (Jangid *et al.* 2009) and are carbonate poor (Tsy-pin & Macpherson, 2012). The soil was imported from USA under the guidelines and licensing of DEFRA. Technical and administrative impediments allowed the purchase of only 20 kg of soil, thus in order to fill all 48 lysimeters, sand was used to make up to the remaining volume. The C₄ soil was sieved through a 4 mm mesh and any visible roots, stones and debris removed. Using two big buckets the 20 kg of soil was mixed with 100 kg of acid-washed sand.

At the bottom of each lysimeter a layer of gravel was placed, to allow better leachate flow and to stop the soil from washing away through direct contact with the hole made for drainage. A total of 120 kg of gravel was used for all 48 lysimeters. The gravel (B&Q, Gravel Natural, 20mm) used for this experiment was chosen because it was described as clean/washed by the provider. However, in order to ensure minimum influence to the leachate draining through the gravel, all 120 kg were washed three times with deionised water. This was achieved one bucket at a time, and then left to dry before filling the lysimeters.

To further stop unnecessary soil loss, two meshes were placed between some of the layers in the lysimeter. A small 2 cm diameter circle of the 0.5 mm mesh, was placed at the bottom of the lysimeter, between the drainage hole and gravel. A 20 cm diameter circle of the 0.5 mm mesh, was placed between the gravel and the first layer of sand. In the end, each lysimeter consisted of the following layers from bottom to the top: mesh, 2.5 kg gravel, mesh, 0.3 kg sand (100 %), 2.3 kg soil-sand mix (20-80 %) and 150 g soil (100 %); the top layer designed to mimic a more organic-rich layer existing in nature (Figure 4.1).

Once the soil was prepared and placed in the lysimeters, 5 g of seeds were sown onto the soil surface of selected lysimeters and watered. The grass chosen for this experiment was *Festuca ovina* (sheep's fescue), because of its drought-resistant nature and the capability to thrive in poor, well-drained mineral soil, making it able to grow and develop well in laboratory conditions, under a regulated water regime. Sheep's fescue is also known to develop symbioses with mycorrhizal fungi, which increase the absorption of water and nutrients. The symbiosis also makes every plant interconnected with the surrounding plants, making possible the exchange of nutrients between plants far from each other (van der Heijden *et al.*, 1998).

The seeds started to germinate approximately one week after sowing.

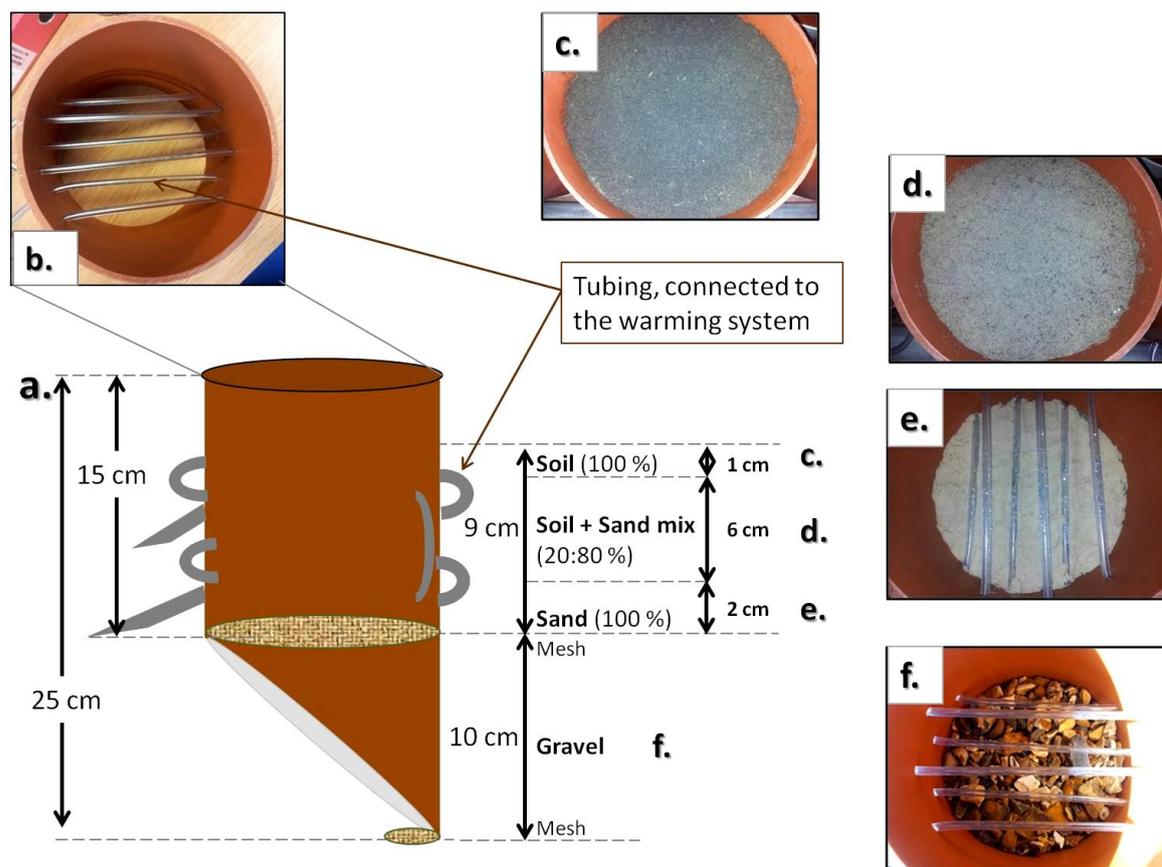


Figure 4.1. Schematic diagram of the lysimeter design, illustrating components, dimensions and layers: the side view of a lysimeter (a), the tubing system staggered on two levels inside each lysimeter (b) and the soil profile layers (c – 100% soil; d – 20:80% soil + sand mix; e – 100% sand and f – gravel).

4.5.1.4. Water addition regime

During the entire duration of the experiment, the quantity of water received by each lysimeter was controlled and calculated according to the changing demands of the soil-vegetation system. All the lysimeters were watered using the same amount of deionised water, three times a week. Figure C3-1 (Appendix C) shows the changes in water addition rates during the experiment. The alterations of water addition rates were necessary due to the increased demand from the growing biomass in the planted lysimeters and later because of increased evaporation in the warmed lysimeters.

4.5.2. Treatment manipulations

Subsequent to the experimental design described in section 4.1, the lysimeters received different treatments described hereafter, following the schedule illustrated in Figure 4.2. The complete description of each factorial treatment assigned to the 48 lysimeters is represented in Figure 4.4.

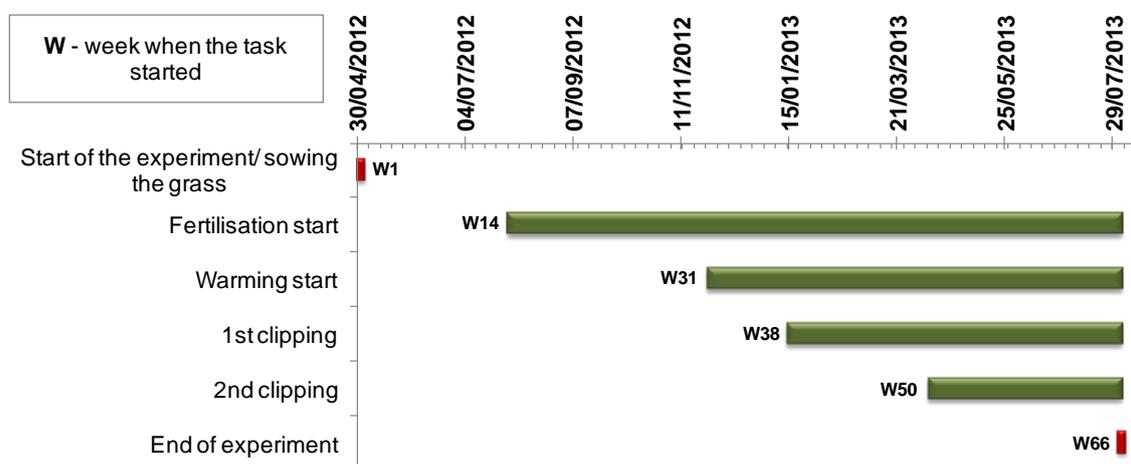


Figure 4.2 The timeline of the experiment, illustrating when each treatment started to be applied

4.5.2.1. Nutrient additions

The first treatment applied to the lysimeters was nutrient additions in the form of ‘All-purpose plant food’ from Phostrogen, with a NPK ratio of 14:10:27. The fertiliser application rate was 20 g per m² per month, which was applied in two sessions, dissolved in deionised water. This rate was calculated using amended values from literature on nutrient enriched grasslands (Keeler *et al.*, 2009). The nutrient additions for this experiment had two levels: **low (L)**, which only received deionised water with 0 g fertiliser added and **high (H)**, receiving the above calculated rate, both in solution using the same amount of water.

4.5.2.2. Warming

The second treatment manipulation was warming. This was applied in two levels: not warmed or **ambient (A)** and **warmed (W)**. Half of the lysimeters in each block were left at room temperature and the other half was warmed up to 5°C above the control soil temperature (Figure 4.3). This was accomplished by using the system described in section 4.5.1.1.

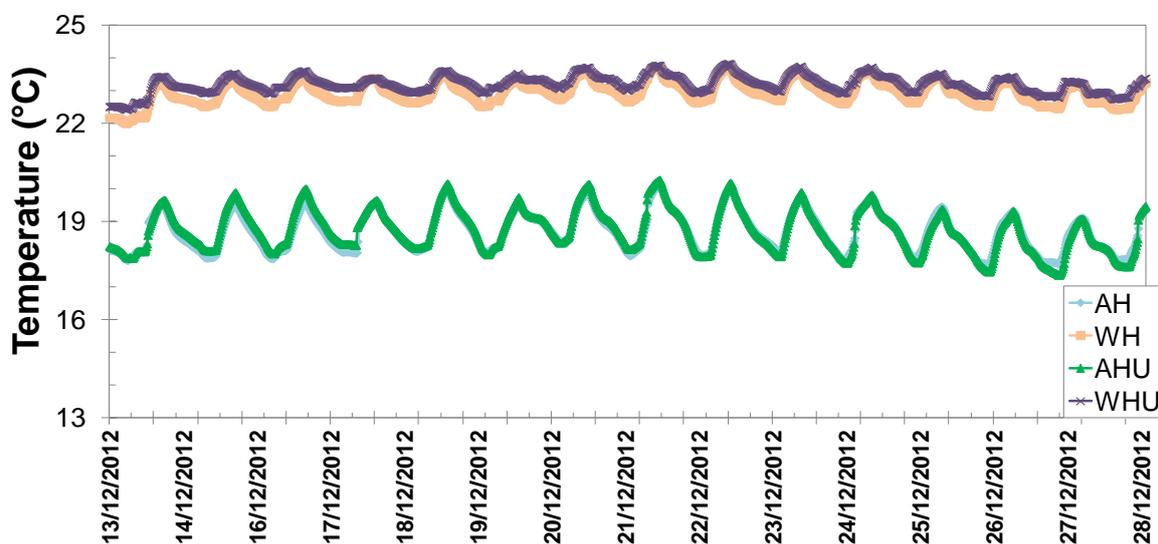


Figure 4.3. Temperature difference between ambient and warmed lysimeters (approx. 5°C) sampled from data collected using temperature loggers. The purple line (planted lysimeter – e.g. WHU) and pale orange line (soil only lysimeter – e.g. WH) show that warming was applied, and the green line (planted lysimeter – e.g. AHU) and the pale blue line (soil only lysimeter – e.g. AH) represent the lysimeters at ambient temperature.

4.5.2.3. Clipping

The third treatment applied was clipping of the above ground biomass, once the plants had to a height of at least 10 cm (exact heights are presented in Table 4.2). For half of the lysimeters in each block the grass was **clipped (C)** and the other half was left **unclipped (U)**. In the clipped lysimeters, half of the canopy height was cut and the litter returned to the lysimeter. This manipulation was applied only twice for the duration of the experiment; once in January 2013 and the second time in April 2013.

Details of the biomass cut and returned to the lysimeters, in each cutting session, are presented in Table 4.2. Only the height of the canopy was considered before cutting, and no weight measurements of the material returned to the lysimeters were made.

Table 4.2. Above-ground biomass height before and after the 2 clipping sessions (14.01.2013 and 09.04.2013)

Nutrient addition	1 ST CLIPPING			2 ND CLIPPING		
	Treatment code	Initial height (cm)	Final height (cm)	Treatment code	Initial height (cm)	Final height (cm)
high nutrient additions	AHC	30	15	AHC	17	11
	WHC	32	16	WHC	17	13
no nutrient additions	ALC	11	5	ALC	5	3
	WLC	12	6	WLC	6	4

4.5.2.4. *Soil only lysimeters*

A fourth treatment was represented by the soil only lysimeters, which received all of the above manipulations except for the clipping. Later in the experiment, because of the high nutrient addition and the lack of competition from plants, mosses started to develop on the surface of the soil. Thus, a decision was made to cover all soil only lysimeters and reduce the moss development. Although a sterilised soil, might have helped reduce the proliferation of mosses, the microbial activity would have been affected too; influencing the soil respiration fluxes and thus changing the results. Also, a sterilised soil could not mimic normal ecosystem conditions, which were hoped to be captured within this experiment, but under controlled conditions.

4.5.2.5. *Pesticide addition*

In February 2013, ten months after the start of the experiment, because of the warm, light and favourable conditions the high nutrient planted lysimeters became infested with aphids (*Aphidoidea* sp.) and springtails (*Collembola* sp.), present on the soil, canopy and in the leachate. The aphids began to deposit a sticky residue on the canopy and when they died they either remained stuck to the grass leaves, fell on the soil or in the leachate beakers. This made it difficult to obtain accurate CO₂ flux readings, without accounting for their respiration, and interfered with the leachate analysis.

Professional help was sought and the next necessary step was spraying the lysimeters with an insecticide. In order to prevent a new treatment being added to only some of the lysimeters, the same amount of insecticide was sprayed in all 48 lysimeters. Following the expert advice, the solution used was PROVADO ULTIMATE BUG KILLER READY TO USE (Bayer CropScience Limited, UK). The spraying took place at the end of March 2013.

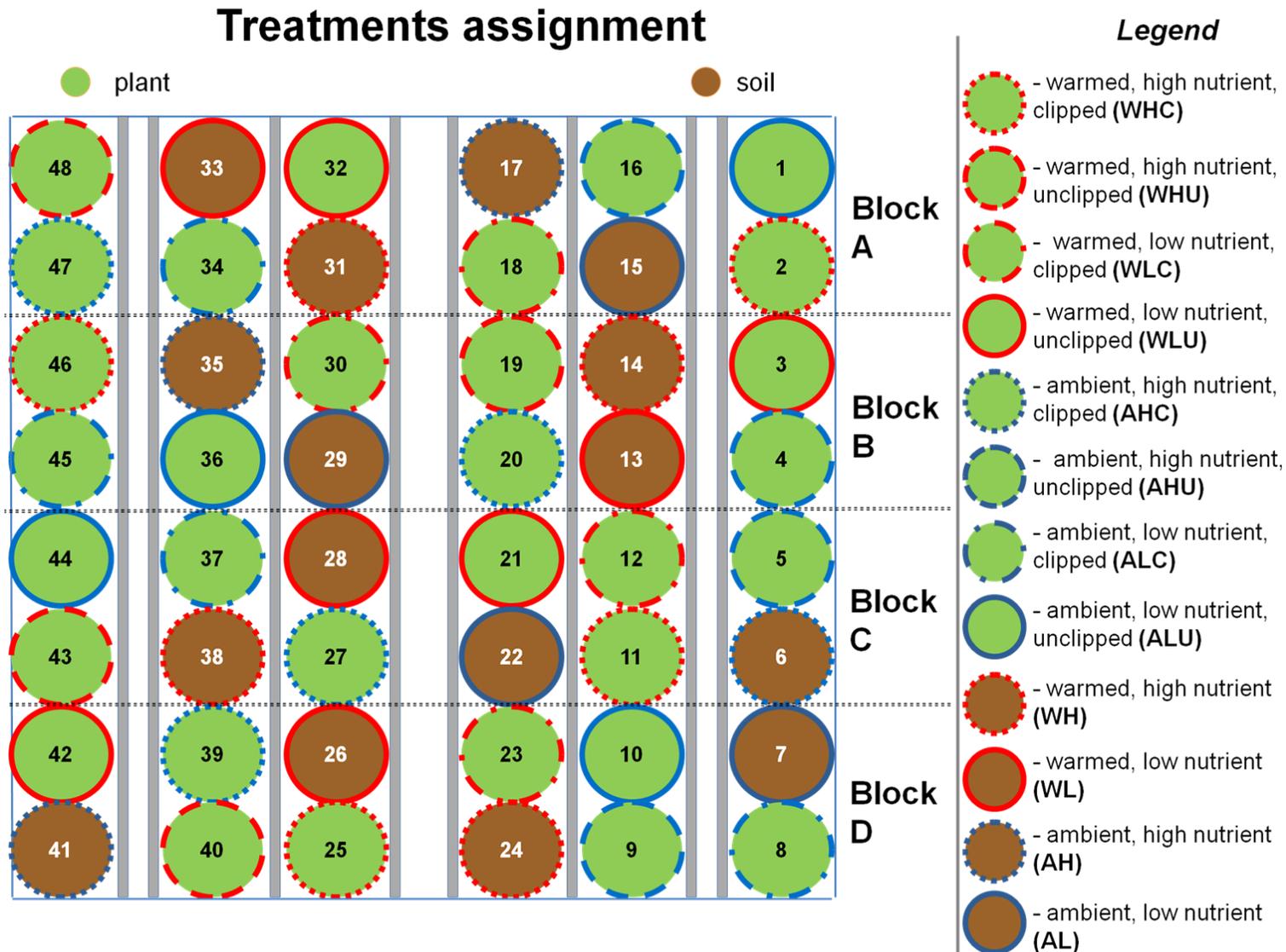


Figure 4.4. Schematic representation of the experimental layout and treatment assignment in each block (A, B, C, D). The green-filled circles represent the 32 lysimeters where plants were grown and the brown-filled circles represent the 16 lysimeters containing bare soil only. The blue circumference indicates that the lysimeters were left at ambient temperature and the red colour circumference indicates warming to approximately 5°C above ambient temperatures. The different dashed circles circumferences represent the nutrient levels for soil only lysimeters (dotted for high N and continuous for low N) and for planted lysimeters it represents nutrient level and whether clipping was applied or not (dotted for high N and clipped canopy, dashed for high N and unclipped canopy, long dash and dot for low N and clipped canopy and continuous line for low N and unclipped canopy).

4.5.3. Data collection and analysis

4.5.3.1. Soil temperature and moisture measurements

The soil temperature and moisture were assessed using a thermometer (Salter Gourmet Digital Meat Thermometer, Tonbridge, UK) and respectively a 4-pin moisture probe (ThetaProbe Soil Moisture Sensor - ML2x, Cambridge, UK). These measurements were taken at the soil surface and 5 cm deep, once a week at first and then monthly. Initially, moisture in particular was monitored closely, in order to adapt the watering frequency and ensure reduced fluctuations. Then, once the seeds had germinated and started growing, measurements were taken every few weeks to keep the soil disturbance to a minimum. An example of soil moisture decline after warming was applied is illustrated in Figure 4.5.

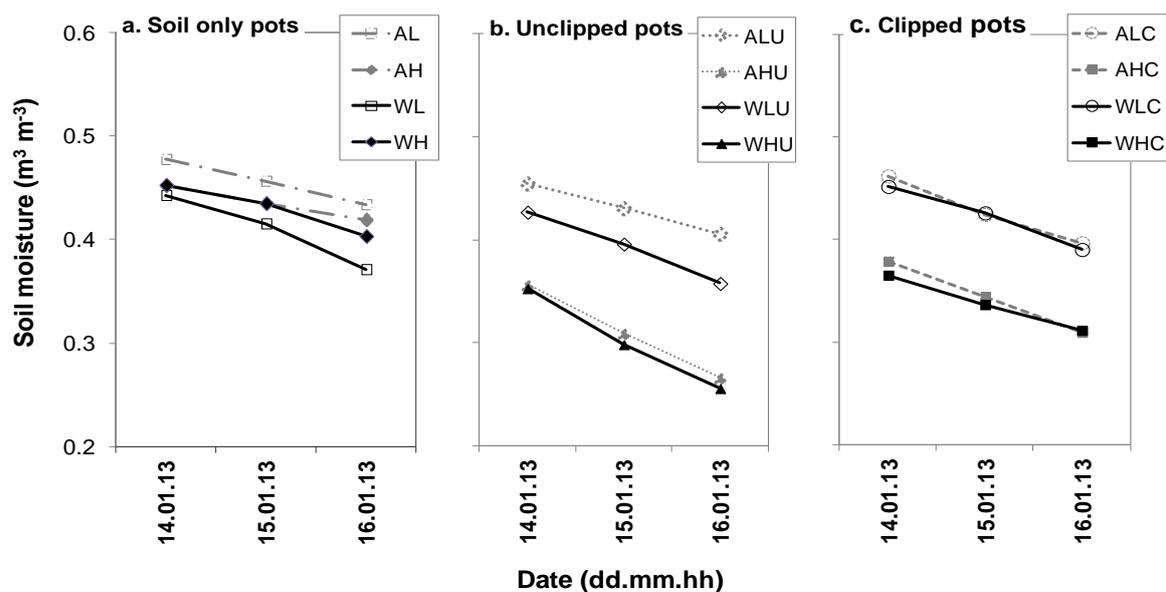


Figure 4.5. Soil moisture decline from the watering day (14 January 2013) until two days later (16 January 2013)

In September 2012, approximately three months before the warming started, additional continuous soil temperature measurements were initiated using Tinytag data loggers (TGP-4020) connected to a Thermistor Probe PB-5001-1M5 (from Gemini Data Loggers Ltd, West Sussex, UK). Due to reduced equipment availability, only four treatments were monitored (two soil only and two planted lysimeters) at ambient (AH & AHU) and warmed (WH & WHU). The loggers were left inserted in the soil of the selected lysimeters and took continuous

measurements every 15 minutes for the remainder of the experiment until August 2013 (12 months).

4.5.3.2. *CO₂ flux measurements*

The main data collected during this experiment consisted of CO₂ flux measurements taken from each lysimeter. These measurements were made using a LiCor system (LiCor, Nebraska, USA), composed of a LI-8100A Control Unit (ACU) attached to a LI-8150 Multiplexer, which enabled two transparent automated chambers to be connected and allowing two lysimeters to be monitored at one time. The chambers were suspended above each lysimeter and sustained by an extension of the original frame. Later in the experiment when the canopy height increased, two additional 40 cm high collars were built from a 20 cm diameter Perspex pipe. These additional transparent collars were necessary in order to reduce the shading and disturbance to the canopy and they were attached to the lysimeter using a 4 cm wide rubber band as illustrated in Picture 4.1. In order to avoid the short-term interference from mechanical disturbance on soil stability and allowing the seeds to germinate, the first measurements were taken six weeks after the seeds were sown. This is in line with recommendations from other studies where measurements started three months or 100 days later (Jaoudé *et al.*, 2011, Polley *et al.*, 1995).

The CO₂ fluxes were measured in the day light and at night time after the photosynthetic activity had ceased, in order to calculate net ecosystem exchange (NEE), ecosystem respiration (ER) and ecosystem photosynthesis. During the first 20 weeks, measurements were made only during the day, recording photosynthetic rates, using just one chamber (due to some technical issues). In week 27 the first night time measurements commenced. Starting with week 30 both chambers were available for alternating measurements and thereafter a total of 10 day light and night time measurement sets were taken until the end of the experiment. During daylight, fluxes from the planted lysimeters were measured by fitting the additional transparent collars allowing photosynthesis to take place. For the soil only lysimeters, both the transparent chambers and additional collars were shaded to allow only respiration to be measured and not the photosynthesis of the

moss and algae that grew in the high nutrient lysimeters. In addition to the day light and night time fluxes, between measurements, the chambers were both set to take continuous measurements from two chosen lysimeters. The purpose of these measurements was to monitor the diurnal variations in the CO₂ efflux, for each of the chosen treatments, as well as the impacts of any background moisture decline and diurnal temperature variations. The two lysimeters, chosen at one particular time, alternated so that the effect of nutrient, warming and above-ground biomass could be assessed in comparison to ambient lysimeters.



Picture 4.1. Illustration of the LI-Cor and lysimeter system used to record ecosystem CO₂ flux measurements.

4.5.3.3. *Leachate collection and analysis*

The excess water from each lysimeter was collected in 500 ml plastic beakers, placed in the trays under the lysimeters. The leachate was collected daily and then combined to produce one sample for each week. Pending analysis the samples were stored at 5-6 °C; the pre-set temperature for the walk-in-fridge, in the Geography Department.

a. Nutrient analysis

During the entire length of the experiment the weekly store leachate samples were analysed for nutrients: nitrogen (N) and phosphorus (P). After measuring the volume of leachate produced by each lysimeter, the samples were run through the auto-analyser machine to determine the two nutrients in the form of phosphate (PO_4^{3-}) and nitrate (NO_3^-), in parts per million (ppm).

The phosphate (PO_4^{3-}) was determined by colorimetric method, Murphy and Riley (1962), and blue colour is formed by reaction of orthophosphate, molybdate ion and antimony ion, followed by reduction with ascorbic acid at a pH of 1. The reduced blue phospho-molybdenum complex was then determined colorimetrically at 880 nm by a detector.

Nitrate (NO_3^-) was analysed via the diazo reaction based on the methods of Armstrong *et al.*, (1967) and Grasshoff (1983). This automated procedure involves reduction of nitrate to nitrite by a copper-cadmium reductor column. The nitrite then reacts with sulfanilamide under acidic conditions to form a diazo compound, which then couples with N-1-naphthylethylene diamine dihydrochloride to form a purple azo dye. The concentration was then determined colorimetrically at 550 nm.

b. Cation analysis

After every calendar month of the experiment, 4-5 weeks' worth of leachate was combined and analysed for cations: Ca^{2+} , K^+ , Mg^{2+} , and Na^+ . The monthly leachate quantity was measured and samples were run through the ICP-AAS machine for each element.

4.5.3.4. *Soil, gas and vegetation sampling and analysis*

The soil was sampled before the start of the experiment from the soil-sand mix used in all the lysimeters and after the completion of the experiment, as cores from all 48 lysimeters after removing the above ground biomass. All the soil samples were then dried, sieved and ground to a fine powder ready to be analysed for organic matter content, carbon stock and $\delta^{13}\text{C}$. The above ground biomass was collected at the end of the experiment. The vegetation was also dried and ground finely in preparation for analyses of total organic carbon, nitrogen, cations and yield.

Samples of respired CO_2 were taken on two occasions for $\delta^{13}\text{C}$ analyses. The gas samples were obtained using the sampling loop illustrated in Figure 4.6. This comprised of a 20 cm diameter PVC collar, 30 cm high and closed at one end placed over the selected lysimeters, connected via PVC tubing to an IRGA (EGM-4; PP Systems, Amesbury, U.S.A.), used to monitor the CO_2 concentrations inside the chamber. Once the concentration reached almost double the atmospheric value, the air in the chamber was flushed through a soda lime scrubber introduced in the loop, until only half of the CO_2 produced remained. This insured that at least half of the CO_2 originating from laboratory air was removed, leaving approximately 20 % of atmospheric CO_2 in the sample. Next, the system was allowed to build up CO_2 again close to 1000 ppm to ensure values within the detection limits of the mass spectrometer used for analysis. After that, the gas was sampled using a syringe and flushed into a 25 ml exetainer.

a. Organic matter content

The organic matter content of the initial soil and the samples collected after the completion of the experiment was determined by the loss on ignition method.

b. Total carbon content and carbon stock

The total carbon content was determined by C/N analysis of soil and vegetation samples. Soil carbon stock was then calculated using the depth of the soil layer in each lysimeter.

c. Isotopic analysis

Stable carbon isotope analysis was carried out in the laboratories of the James Hutton Institute (JHI), Scotland. Ground soil samples were weighed into tin capsules and placed in a sealed 96 well plate, prior to transport to JHI together with the exetainers containing the gas samples. In their laboratories, $\delta^{13}\text{C}$ in the soil was determined with a Flash EA 1112 Series Elemental Analyser (EA) connected via a Conflo III to a Delta^{Plus} XP isotope ratio mass spectrometer. The carbon isotopes of carbon dioxide in the gas samples was determined by a Gasbench II connected to a Delta^{Plus} Advantage isotope ratio mass spectrometer. All instruments used were Thermo Finnigan, Bremen, Germany.

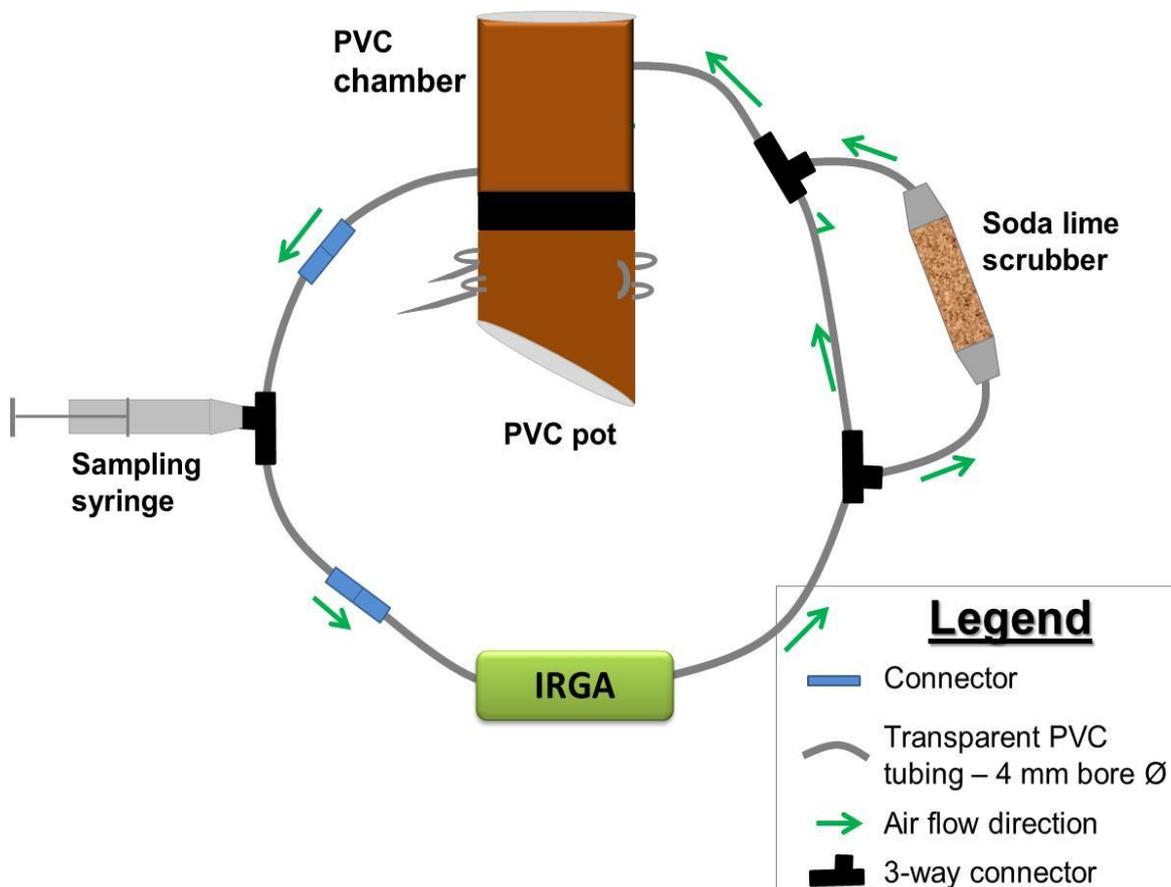


Figure 4.6. Schematic diagram of the sampling loop used to extract the gas samples to be analysed by $\delta^{13}\text{C}$

4.5.4. Calculations and statistical analysis

The entire data recorded collected during this experiment was organised in Microsoft Excel (Microsoft Office Professional Plus 2010, ©2010 Microsoft Corporation), where the necessary corrections and calculations were made. After coding, the data were analysed statistically with SPSS (IBM® SPSS® Statistics 20). First the data was tested for normality. Homogeneity of variance was tested using Levene's test. Data were transformed where assumptions were broken allowing for parametric data analysis. One-way/ two-way/ three-way ANOVAs were used to determine the significant differences between different treatments and their factor levels. Where overall significant effects were observed, Tukey and LSD post hoc tests were employed to determine which factor levels differ. All the graphs and figures used for illustration were created in Excel.

One-way ANOVA was only used to test the overall differences between all 12 treatments. Two-way ANOVAs were used to test the main and interactive effects of any two factors (fertilisation, warming and clipping) at a time on the CO₂ fluxes, nutrient and cations loss and final gas, soil and vegetation parameters. The interaction effect between all three factors (fertilisation, warming and clipping) was determined by three-way ANOVA. The change with time of all these factors and the interactions between them was tested using repeated measurements ANOVA.

The CO₂ fluxes measured were separated into net ecosystem exchange (NEE), ecosystem respiration (ER) and gross primary productivity (GPP) as follows:

$$[\text{Eq. 16}] \quad \mathbf{NEE} = \text{day light fluxes}$$

$$[\text{Eq. 17}] \quad \mathbf{ER} = \text{night time fluxes}$$

$$[\text{Eq. 18}] \quad \mathbf{GPP} = \text{ER} - \text{NEE}$$

The effect of warming, nutrient additions and clipping on ER and GPP was compared between treatment and control with a paired *t*-test.

The carbon sequestered by the ecosystem as net primary production was calculated as the difference between uptake and loss:

$$[\text{Eq. 19}] \quad \mathbf{NPP} = \text{GPP} - \text{ER}$$

4.6. RESULTS

4.6.1. Baseline measurements

4.6.1.1. *Soil temperature and moisture*

The soil moisture and temperature data illustrates the changes planned as part of the original experimental design. The amount of water added to the lysimeters, changed through time due to demand from the growing biomass and the increased soil temperatures due to warming applied to half of the lysimeters. The treatments applied during the experiment, affected the soil temperature (Figure 4.7) and moisture (Figure 4.8) as follows.

Soil temperature data collected using the automated probe loggers, were limited to four of the 12 treatments and represent both the soil only lysimeters (ambient – AH; warmed – WH) and the planted lysimeters (ambient – AHU; warmed – WHU). Before December 2012 when the warming was applied, there is no significant difference between any of these four treatments (Figure 4.7). After warming started, the treatments separate as expected between warmed and control with an approximate difference of 3-4 °C; but no significant difference is recorded between the soil only and planted lysimeters in either the warmed or the control groups.

From December 2012 to March 2013, the ambient temperatures were relatively constant around 18-20 °C, making it easy to maintain a 3-4 degrees difference between control and warmed. Starting with April 2013, the rising ambient temperatures increased the recorded levels from the control lysimeters, forcing a further rise in the warmed lysimeters to maintain the necessary difference. At the beginning of July 2013, the ambient temperatures raised further, making it difficult to maintain the 3-4 degree difference without creating artefacts in the warmed lysimeters and thus the experiment coming to an end soon after that. The occasional drop in temperature in the warmed lysimeters, observed in Figure 4.7 are due to either planned stops of the warming systems, technical faults with the thermostat or power cuts.

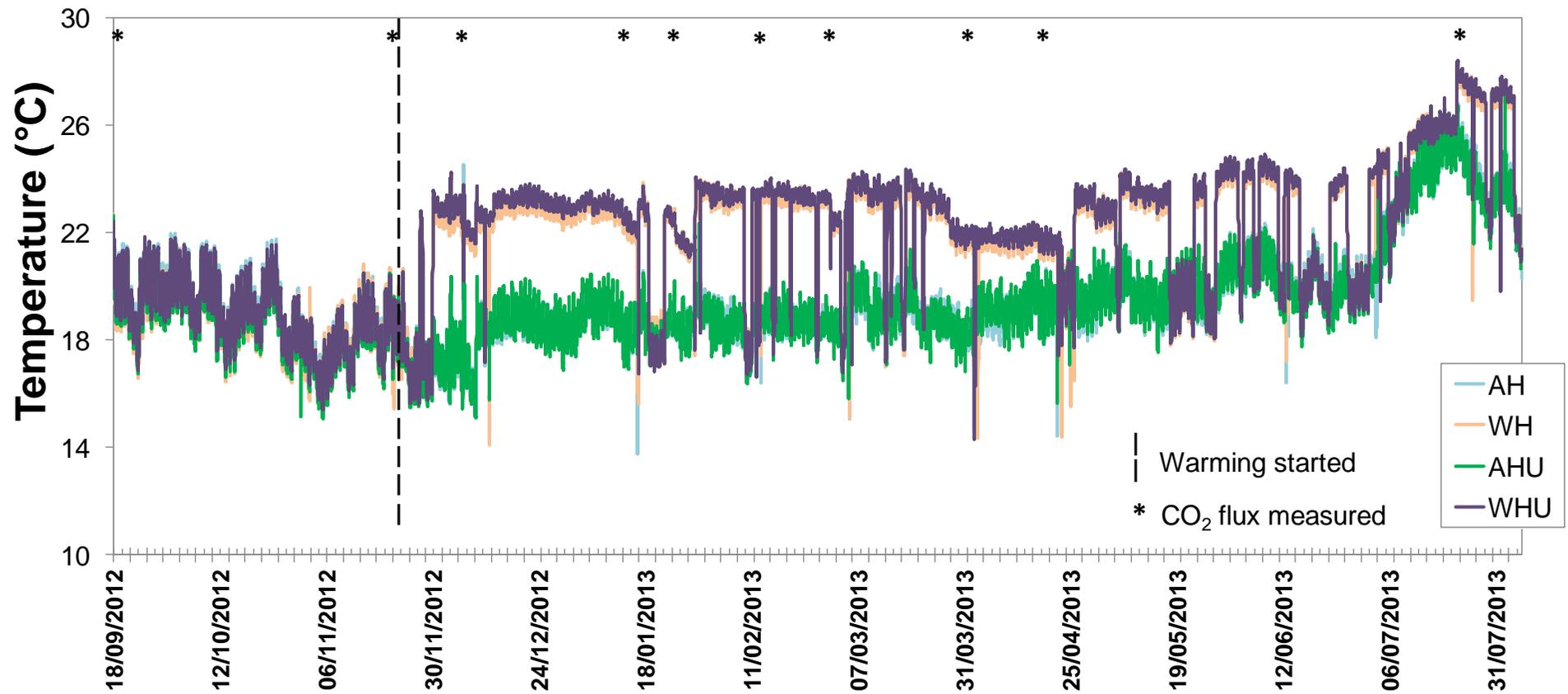


Figure 4.7. Temperature changes and differences between four treatments before and after the warming was applied. The stars (*) indicate the dates coinciding with the CO₂ efflux measurements.

Contrary to the temperature data, the moisture measurements were made using a manual probe, thus less data were recorded. The measurements were separated between the lysimeters containing soil only and planted ones: clipped and unclipped. In the soil only lysimeters, soil moisture varied very little during the 15 months of the experiment and no significant differences ($P > 0.05$) can be observed between treatment types. In the planted lysimeters, both clipped and unclipped, an emerging trend indicates lower soil moisture in the high nutrient lysimeters compared to the one without any nutrient additions (Figure 4.8 – a & b). The impact of warming on moisture is marginally different ($P < 0.08$) in the unclipped lysimeters, between the two low nutrient treatments (ALC & WLC), where the ambient lysimeters had higher soil moisture than the warmed.

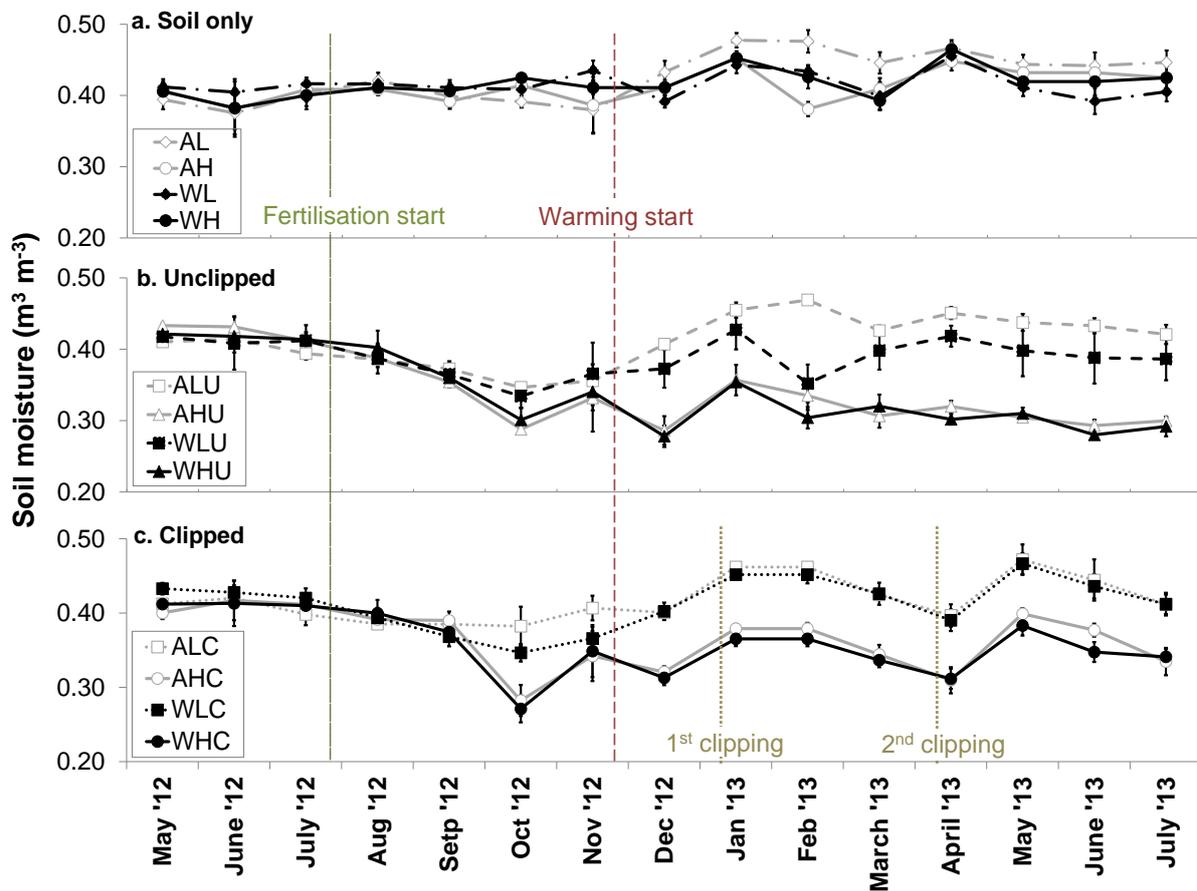


Figure 4.8. Soil moisture timelines for the soil only lysimeters (a.), unclipped planted lysimeters (b.) and clipped planted lysimeters (c.). Comparison is made between the lysimeters with and without added nutrients and between ambient and warmed lysimeters. These can be observed relative to the three main manipulations: nutrient additions (continuous vertical line), warming (dashed vertical line) and clipping (dotted vertical line). Error bars are \pm SE ($n=4$).

4.6.1.2. Leaching rates

As a consequence of the changes in biomass and the subsequent water addition rates, the leaching rates from each lysimeter changed too. For the soil only lysimeters (Figure 4.9 – a) the most significant difference in leaching rates was observed between the temperature treatments, with higher rates recorded from the warmed lysimeters. While no significant difference ($P > 0.05$) was observed between the nutrient treatments alone. The planted lysimeters recorded a different trend than those without vegetation. Significantly higher ($P < 0.05$) leaching rates were calculated for the lysimeters receiving no nutrient additions, in both clipped and unclipped treatments. At the same time, temperature affected the leaching rates too, presenting higher values for ambient lysimeters, significant in the unclipped treatments ($P < 0.05$) and only marginally significant ($P > 0.09$) for clipped lysimeters. The high nutrient lysimeters lost considerably less water than those without, but no significant difference was observed between warmed and ambient treatments for either of the planted lysimeters.

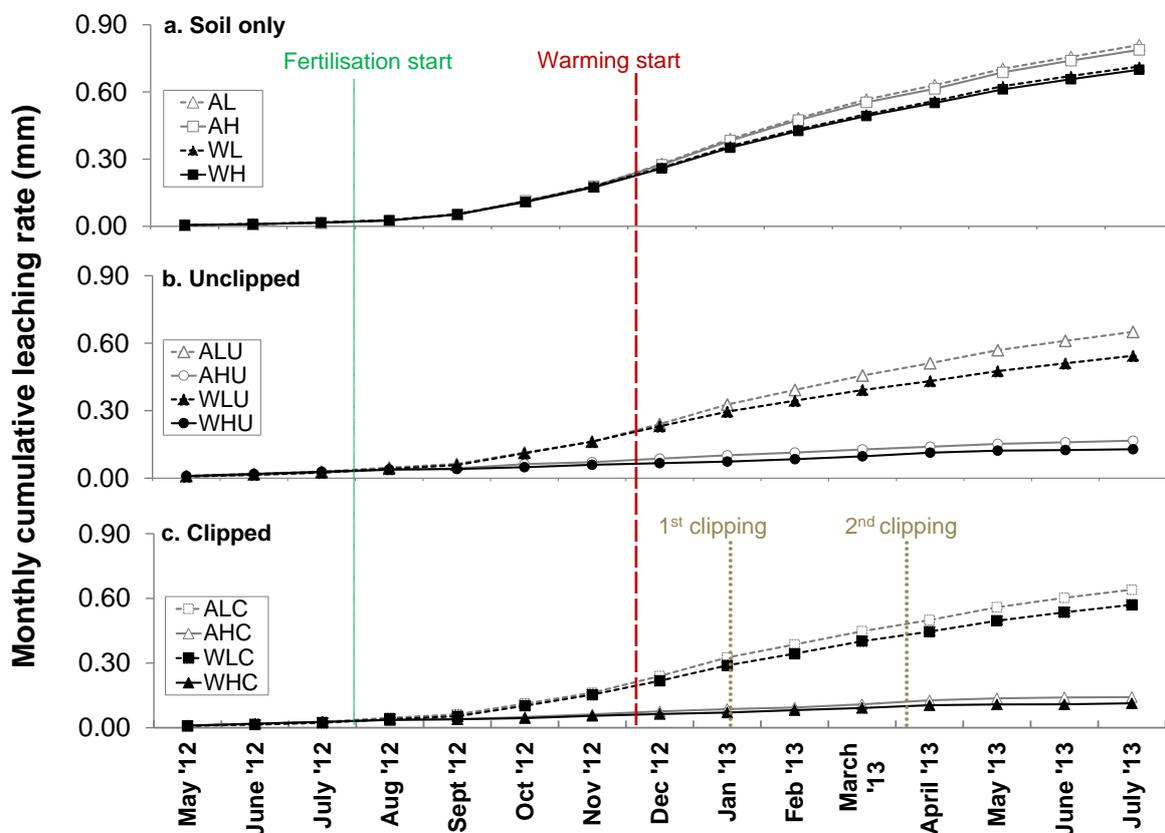


Figure 4.9. Monthly cumulative leaching rates from the soil only lysimeters (a), the unclipped lysimeters (b), and the clipped lysimeters (c).

4.6.2. Ecosystem productivity and parameter variations

The soil fluxes ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) recorded during the day and night were used to calculate the amount of C ($\mu\text{g m}^{-2} \text{ s}^{-1}$) respired or assimilated by the plant and/or soil system in each lysimeter receiving different treatments and manipulations. The data collected from all 12 treatments were separated into the three main manipulations made to the lysimeters: soil only, planted clipped and planted unclipped. Calculations of both ecosystem respiration (ER) and gross primary productivity (GPP) began in October 2012, when night time measurements were made possible due to additional equipment alongside the daytime measurements which started in May 2012. Towards the end of February 2013, the high nutrient planted lysimeters became infested with insects which started to secrete a sticky substance covering the grass. Following expert advice, all the 48 lysimeters were sprayed in March 2013 and became insect-free by the middle of April 2013.

4.6.2.1. Ecosystem respiration (ER)

All the fluxes measured at night were considered to be ecosystem respiration (ER) and are presented in Figure 4.10, relative to the nutrient additions, warming and clipping.

Analysed together, the four soil only treatments (AL, AH, WL and WH) indicated significantly different ($P < 0.028$) ER levels from December 2012 to July 2013, but not in March 2013 ($P > 0.05$). In the soil only lysimeters (Figure 4.10 - a) the effect of warming was significantly different ($P < 0.006$) from the controls, from January 2013 until the end of the experiment, with exceptions in March, June and July 2013 ($P > 0.11$). There was no significant interaction effect recorded between warming and nutrient additions, though after warming was applied, both warmed lysimeter treatments (WL and WH) had slightly higher ER values than their corresponding ambient treatments (AL and AH).

In the planted plots, the separation between low and high nutrient addition lysimeters was more obvious than in the soil only ones. In both the unclipped and

clipped lysimeters, the ER of high nutrient treatments recorded almost twice the magnitude of the low nutrient ones (Figure 4.10 - b and c).

Observations of the unclipped lysimeters (ALU, AHU, WLU and WHU) indicated significantly ($P < 0.015$) higher ER levels in the warmed high nutrient lysimeters compared to the ambient equivalents, in December 2012 and January 2013. From February to April 2013, the difference between warmed and ambient declined due to insect activity and subsequent actions to remove them. Once the effect of the insecticide applied diminished, the ER levels in the high nutrient lysimeters reversed indicating significantly higher levels in the ambient compared to the warmed lysimeters. There was no significant difference observed between the unclipped low nutrient lysimeters (ALU and WLU), though visually the warmed had slightly higher ER levels than the ambient ones (Figure 4.10 – b).

The clipped lysimeters (ALC, AHC, WLC, and WHC) showed similar trends to the unclipped treatments (Figure 4.10 – c). A month after the 1st clipping was applied in January 2013, the high nutrient lysimeters (AHC and WHC) recorded an increase by almost half the magnitude of ER levels before clipping, though no significant difference was shown between warmed and ambient treatments. Just like the unclipped lysimeters, the clipped too inverted levels, showing higher ER in the ambient than in the warmed ones. A small but not significant difference ($P < 0.21$) was observed between the two low nutrient lysimeters (ALC and WLC). The 2nd clipping applied in April 2013 made no further changes, continuing to show no significant differences between treatments until the end of the experiment.

The effect of warming on the ER was additionally tested by 2 tailed *t*-test analysis of the ambient and warmed lysimeters. The results are summarised in Table C2-1 (Appendix C).

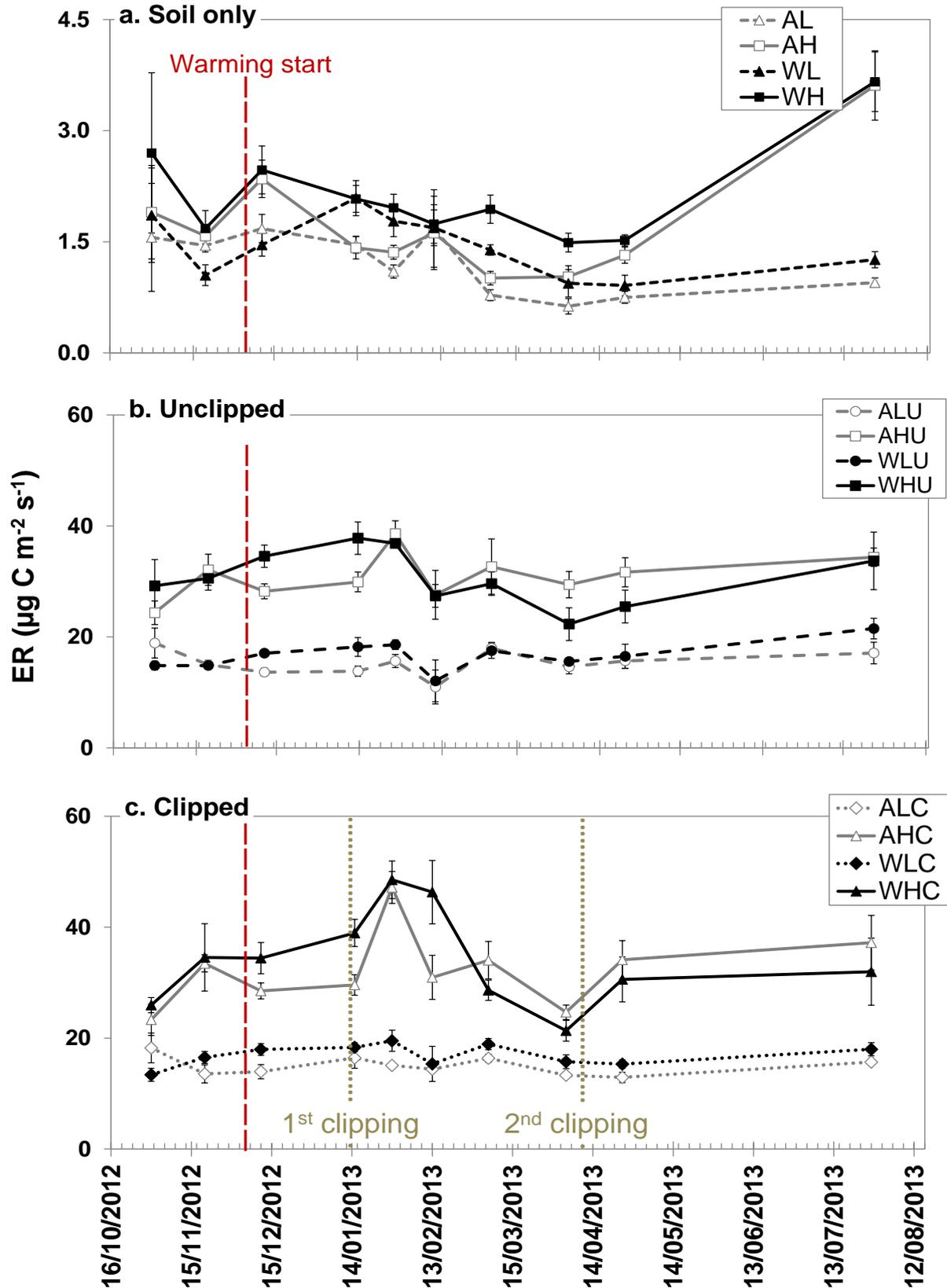


Figure 4.10. Ecosystem respiration (ER) timeline for soil only lysimeters (a.), unclipped planted lysimeters (b.) and clipped planted lysimeters (c.), relative to the main manipulations applied, warming (dashed vertical line) and clipping (dotted vertical lines). Error bars are \pm SE (n=4).

4.6.2.2. *Net ecosystem exchange (NEE)*

Net ecosystem exchange (NEE) was considered equivalent to the CO₂ flux measurements taken during the day. A negative flux represents a net CO₂ uptake by vegetation and positive flux represents net CO₂ release to the atmosphere (Figure 4.11).

The soil only lysimeters (AL, AH, WL, and WH) showed evidence of net release of C (Figure 4.11 – a). The highest release of C was measured in the lysimeter receiving high levels of fertiliser in addition to the warming treatment (WH). This was significantly higher than the results obtained from the ambient lysimeters with (AH) and without (AL) any fertilisation. A significant difference was also revealed between the warmed and ambient lysimeters that had no nutrients added (WL and AL). NEE was higher in the warmed lysimeters for a month after the warming was applied, but the difference declined in February and March and resumed the following month only to decline again thereafter.

However, the planted lysimeters registered a negative NEE, consistent with a net uptake of C by the system (Figure 4.11 – b & c). Both unclipped (ALU, AHU, WLU, and WHU) and clipped (ALC, AHC, WLC, WHC) lysimeters presented a similar C uptake trend, with the nutrient treatment as the main factor differentiating between fluxes. In the unclipped lysimeters, the warming reduced NEE in the high nutrient treatment from January onwards. Significant differences were observed from mid-February, but there was little effect of warming on NEE in the low nutrient lysimeters. Warming had no further effect on the NEE and neither did the clipping. The initial higher C uptake by the lysimeters with a higher nutrient treatment continued after the warming was applied, but the difference declined soon after, while the low nutrient ones maintained a more constant trend throughout.

There was no significant effect of clipping on NEE overall, but the reduction in net uptake in the high nutrient lysimeters occurred earlier in the clipped lysimeters than in the unclipped plots (Figure 4.11).

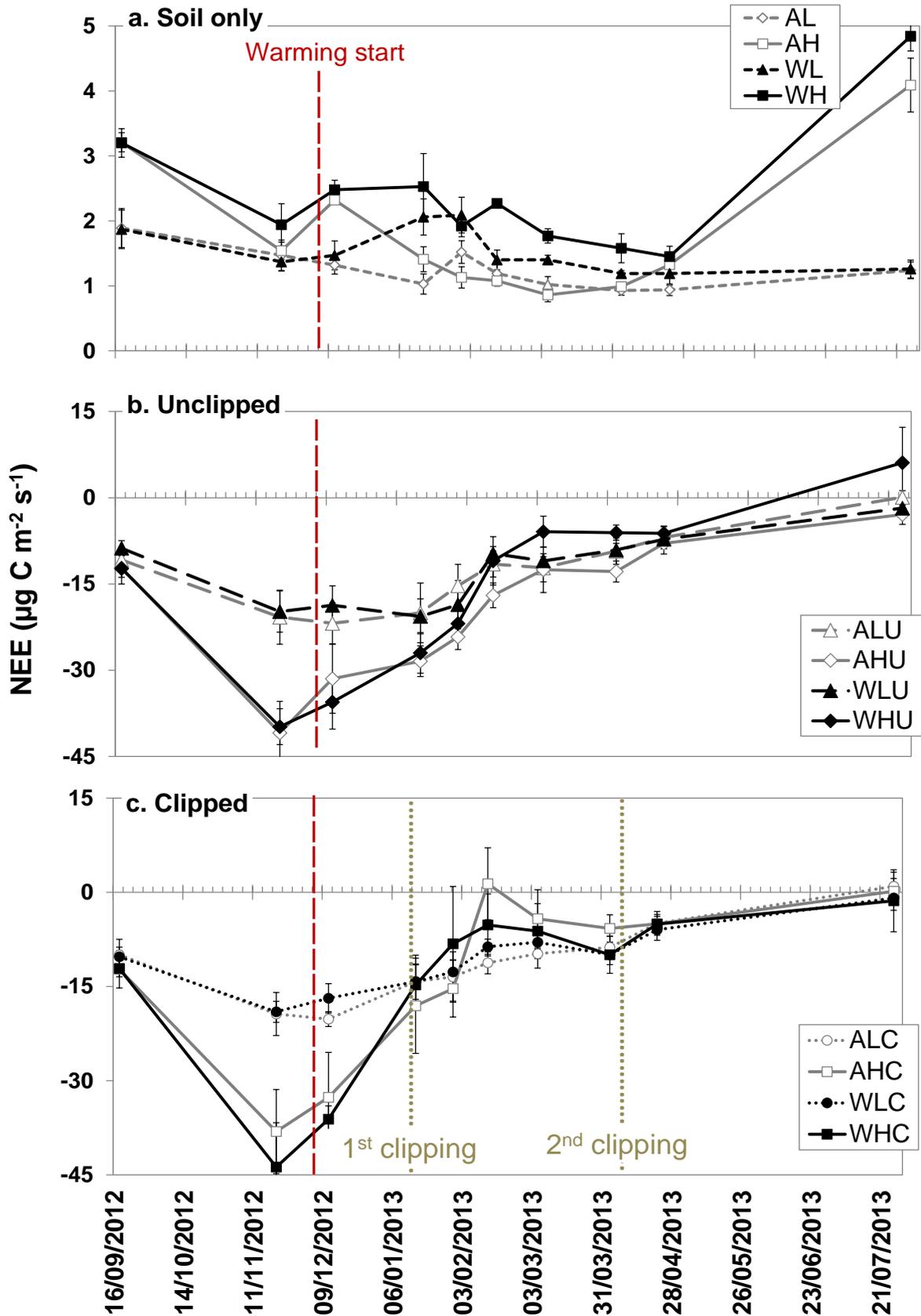


Figure 4.11. Net ecosystem exchange (NEE) timeline for soil only lysimeters (a.), unclipped planted lysimeters (b.) and clipped planted lysimeters (c.), relative to the main manipulations applied, warming (dashed vertical line) and clipping (dotted vertical lines). Error bars are \pm SE (n=4)

4.6.2.3. Gross primary productivity (GPP)

The gross primary productivity (GPP) of the ecosystem under investigation was calculated as the ecosystem respiration (ER) minus the net ecosystem exchange (NEE).

Separated into soil only, unclipped and clipped lysimeters, the GPP data were first transformed, then analysed for statistical differences with the results presented in Figure 4.12. For the soil only lysimeters (AL, AH, WL and WH) the GPP calculated was due to the increase in moss biomass in most lysimeters, especially in those receiving high nutrient additions. There were no statistical differences between the investigated treatments, plus no evident trend emerged during the length of the experiment (Figure 4.12 – a). The planted lysimeters showed similar trends and behaviour to the ER data. The most evident separation between treatments continued to be the level of nutrient added, with the higher nutrient lysimeters having significantly higher GPP values ($P < 0.001$) than those without any nutrient additions.

Following the start of warming in the unclipped lysimeters (ALU, AHU, WLU and WHU), the treatments recorded a marginal difference between warmed and ambient in both the low and high nutrient addition lysimeters (Figure 4.12 – b). Similar to the ER data, starting in February 2013 and until the completion of the experiment, the AHU treatment became marginally higher ($P > 0.118$) than WHU, but this was only significantly different ($P < 0.018$) in May and July 2013. No significant differences were recorded in the low nutrient lysimeters. In the clipped lysimeters (ALC, AHC, WLC and WHC), the only significant difference ($P < 0.001$) was observed between the high and low nutrient addition treatments with no significant differences due to warming (Figure 4.12 – c).

Additional information regarding the influence of warming on the GPP, was obtained by 2 tailed *t*-test analysis of the ambient and warmed lysimeters. The results are summarised in Table C2-1 (Appendix C).

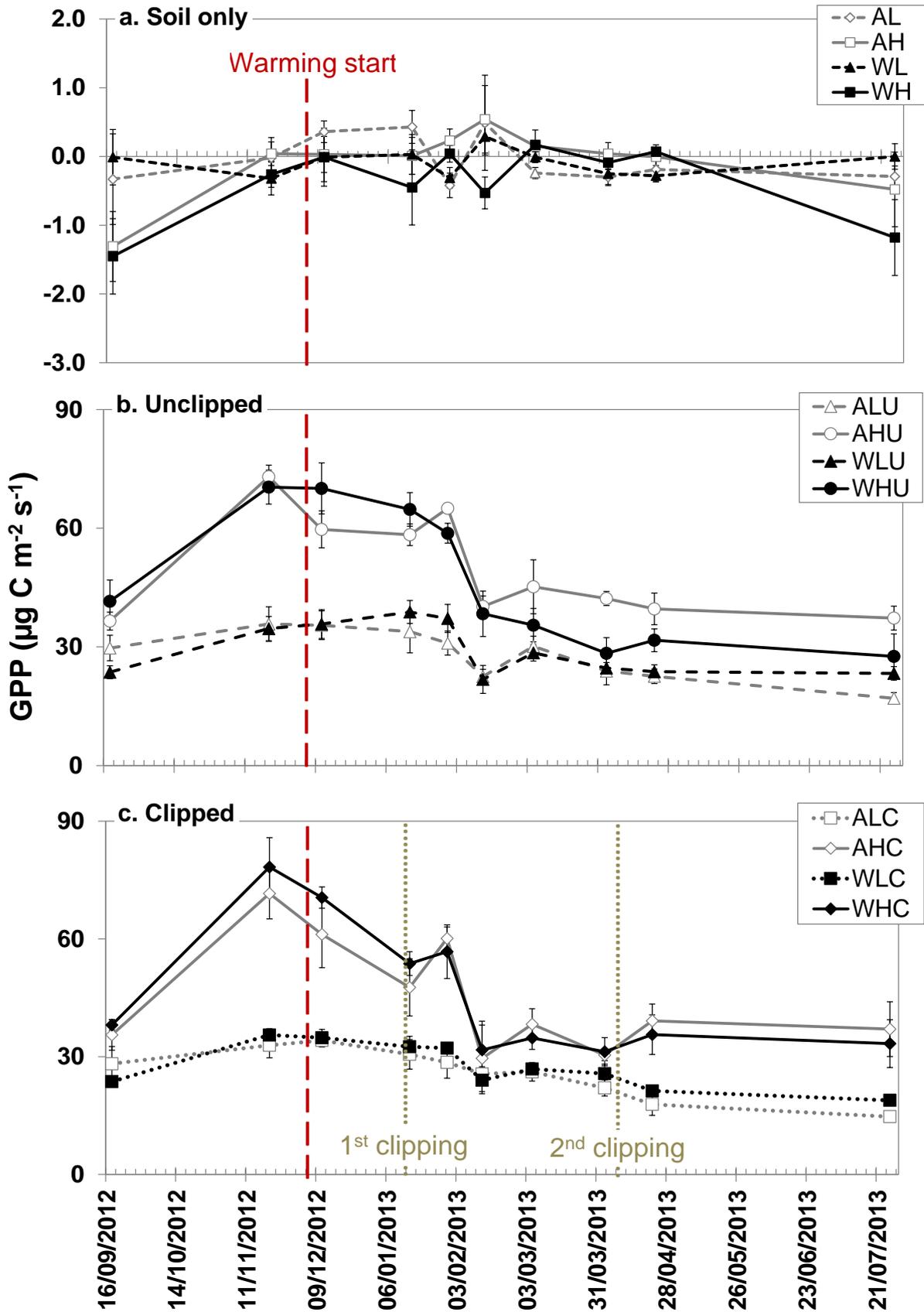


Figure 4.12. Gross primary productivity (GPP) timeline for soil only lysimeters (a.), unclipped planted lysimeters (b.) and clipped planted lysimeters (c.), relative to the main manipulations applied, warming (dashed vertical line) and clipping (dotted vertical lines). Error bars are \pm SE (n=4).

4.6.2.4. Diurnal variation of ecosystem parameters

The continuous ecosystem CO₂ exchange data collected by running simultaneously both chambers, for a day and up to a week, provided important information regarding the influence of nutrient and/or warming on the C fluxes in the long term. Figure 4.13 presents the ecosystem fluxes of the soil only lysimeters before any treatment and manipulations were applied (a) and a month after the start of fertilisation (b).

In the first months after the beginning of the experiment (Figure 4.13 – a) the respiration levels in the soil only lysimeters were very low ($0.2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), and remained the same later in the experiment. Once the fertilisation treatment was applied (Figure 4.13 – b), the magnitude of the respiration spiked almost three fold ($0.6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), also increasing the difference between day and night fluxes from 0.12 to $1.60 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. The additional nutrient in the AH lysimeters also encouraged the development of moss and algae, which contributed to the difference observed in respiration fluxes between day and night. The attempt of killing the moss by covering the soil only lysimeters and reducing the access to light, yielded little visible results. Some of the moss species died in the favour of others that thrived in the dark, thus part of the fluxes recorded in the high nutrient soil only lysimeters can be explained by their presence.

For the planted lysimeters, once warming started in December 2012 and clipping in January 2013, other comparisons between diurnal variations of different level of nutrient treatments were possible (Figure 4.14). Comparisons between ambient and warmed low and high nutrient lysimeters (Figure 4.14 - a) revealed a significant difference ($P < 0.05$) between nutrient levels applied, but not between the two temperature levels ($P > 0.05$), although a difference of almost $4 \text{ }^\circ\text{C}$ was recorded between the two treatments.

When clipped and unclipped low nutrient treatments were compared (Figure 4.14 – b), the ecosystem fluxes indicated no significant difference ($P > 0.05$) between control and warmed, or between clipped and unclipped manipulations. Fluxes retained the same magnitude, of approximately $2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, between all compared treatments during the day and at night.

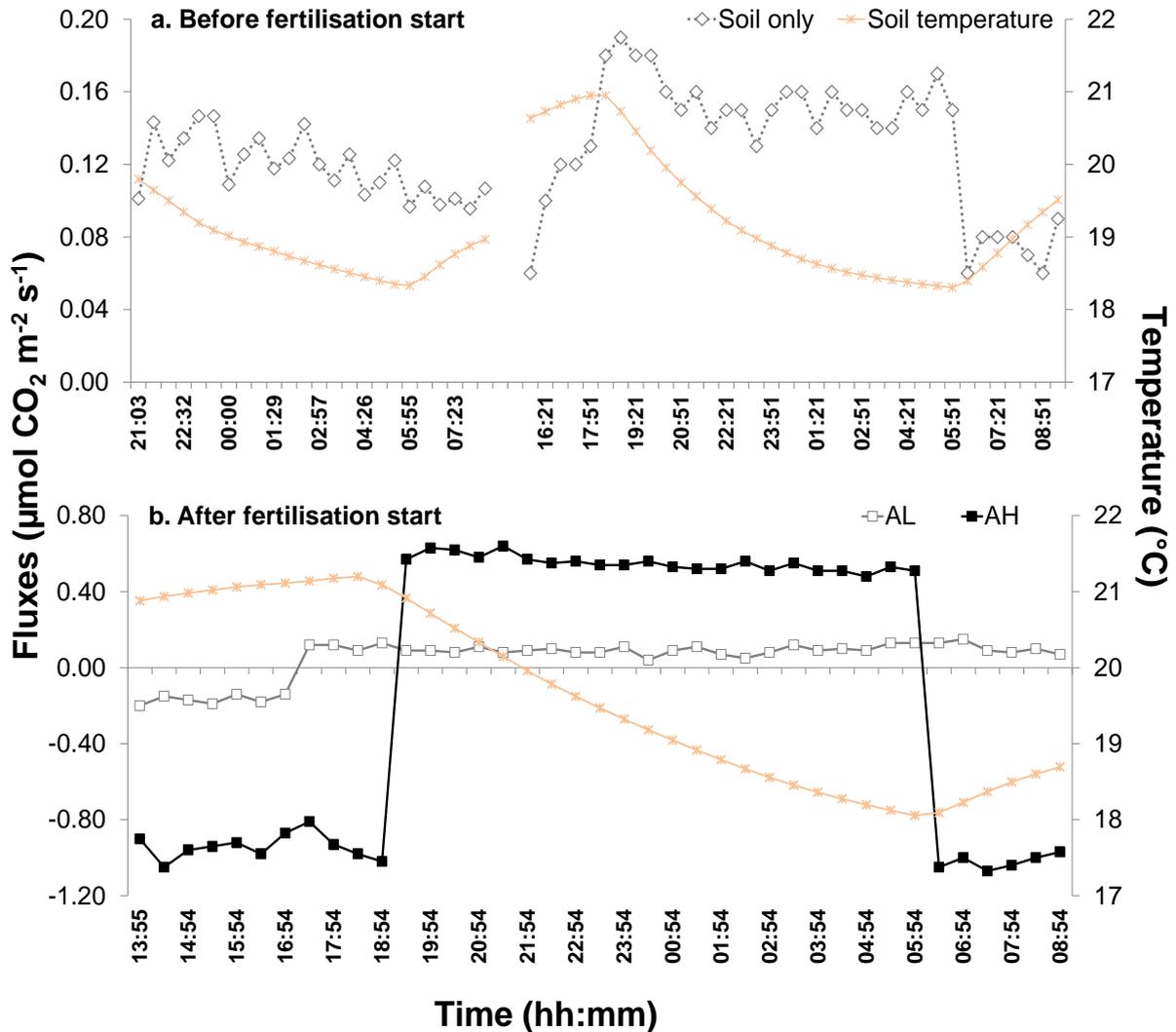


Figure 4.13. Diurnal variation of the ecosystem fluxes in the soil only lysimeters, in June – July 2012 before any manipulations were applied to the lysimeters (a.) and in August 2012 after fertilisation treatment began (b.). Both panels of the graph represent data before warming started in December 2012; the variation in soil temperature, illustrated by the green line, is due to the changes in atmospheric temperature between night and day. The break in the line from panel (a) represents a power cut, thus data could not be used. The sudden shift in the lines is represented by the lights coming on and warming up the air and soil in the lysimeters. The lights were set to switch on at 6 am and switch of at 19 pm.

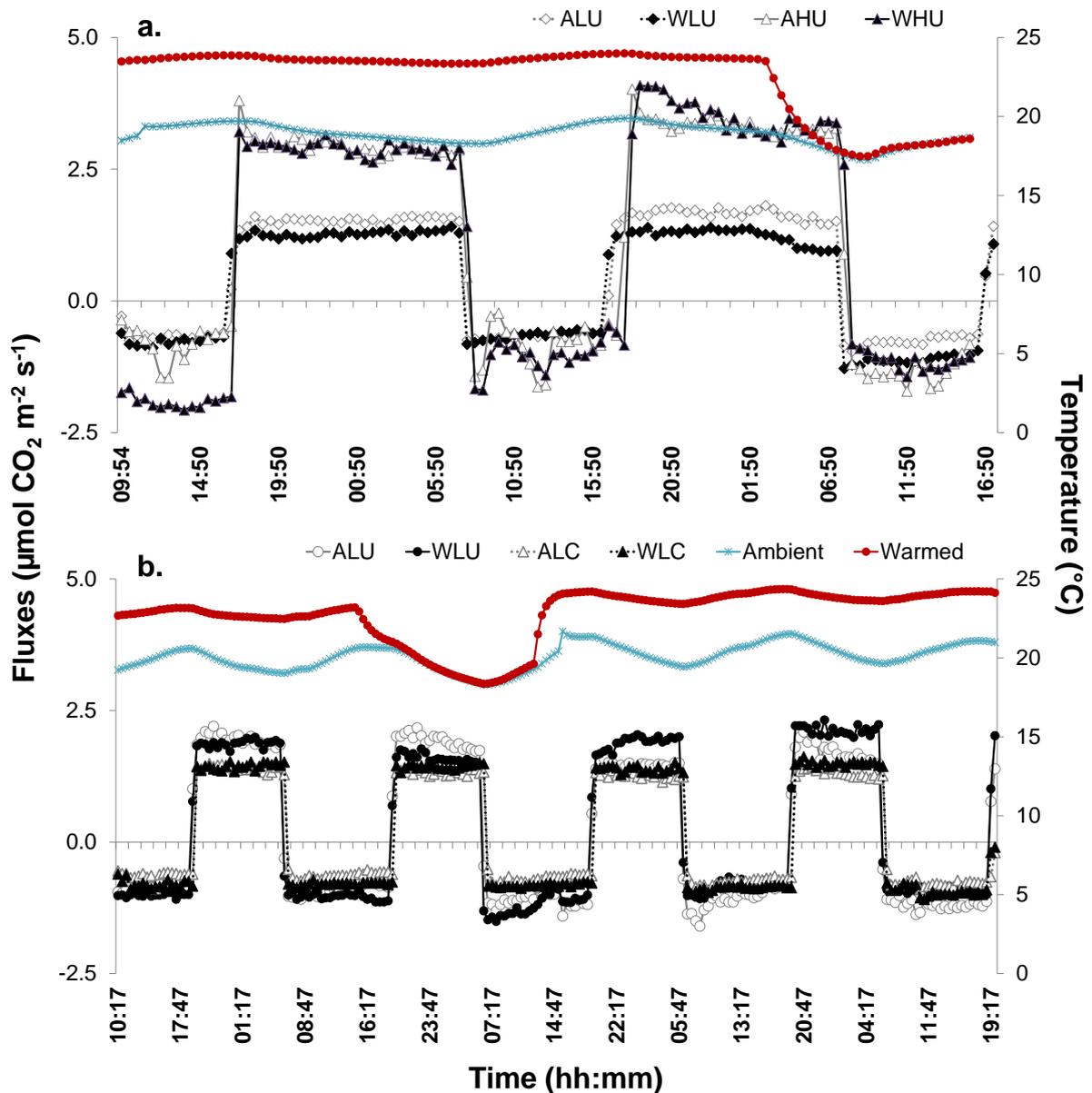


Figure 4.14. Diurnal variations of ecosystem fluxes relative to the soil temperatures recorded in the control (blue lines) and warmed (red lines) lysimeters. The fluxes illustrated represent comparisons between low and high nutrient additions (ALU-WLU & AHU-WHU) in ambient and warmed lysimeters (a.) and between clipped and unclipped (ALC-WLC & ALU&WLU) ambient and warmed lysimeters receiving low nutrient (b.) The dip in the temperature from the warmed lysimeters, was due to technical issues with the thermostat.

4.6.3. Isotopic analyses

4.6.3.1. Gas samples

Isotopic analyses were carried out on the gas samples collected in May 2013 (12 months after the experiment began) and in August 2013 (at the end of the experiment). For the samples collected in May 2013 (Figure 4.15 – a), the results indicate $\delta^{13}\text{C}$ values of -29.54 ‰ for the ambient lysimeters and -28.96 ‰ for the warmed lysimeters. The samples collected in August 2013 (Figure 4.15 – b) had $\delta^{13}\text{C}$ values of approximately -20.73 ‰ for soil only lysimeters and -27.55 ‰ for the planted unclipped lysimeters. For the plant-plots, no significant differences were observed between the warming or nutrient treatments. While efforts were made to remove as much ambient air from the chambers as possible, before sampling, at least 1/3 of the levels recorded are explained by the presence of the ambient CO_2 .

4.6.3.2. Soil samples

The results of the isotopic analysis on the soil-sand mix before the lysimeters were established, indicate an initial $\delta^{13}\text{C}$ value of -14.44 ‰ (Figure 4.16). This value is within the expected range for a C4 soil of -14 ‰ to -10 ‰, as previously reported (Kao and Liu, 2000). If there was a significant quantity of carbonates present in the soil, the values would have been in the range of -4.0 ‰ and -10.0 ‰ as reported in some papers investigating the isotopic signatures of carbonates (Stevenson *et al.*, 2005). After 15 month of experiments and treatment applications, the values in the soil only lysimeters were approximately -14.49 ‰ (Figure 4.16 - a), unclipped planted lysimeters -17.87 ‰ (Figure 4.16 - b) and clipped planted lysimeters -18.40 ‰ (Figure 4.16 - c).

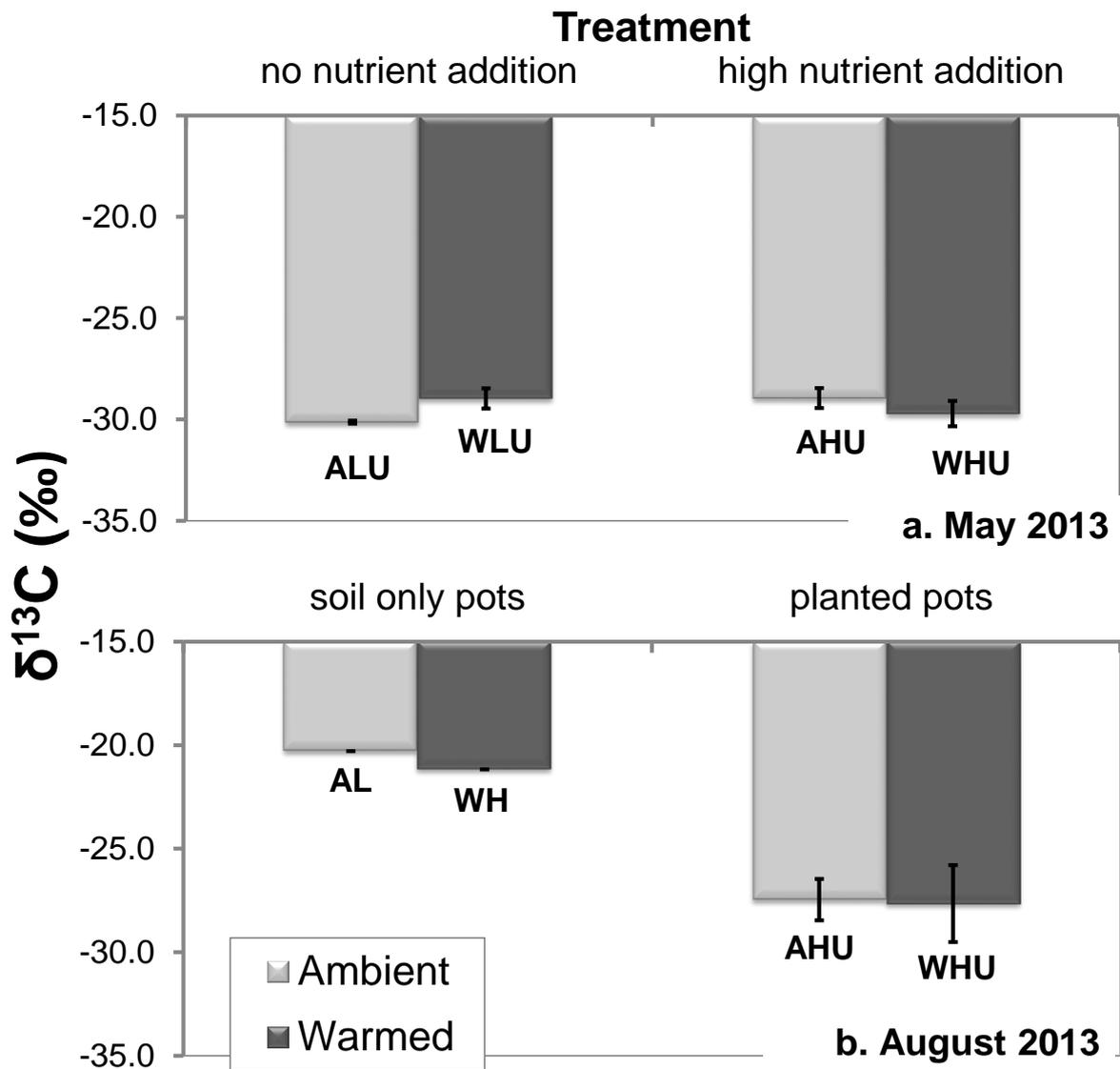


Figure 4.15. Gas sample results for the $\delta^{13}\text{C}$ analysis indicating the levels recorded in May 2013 (a.) and August 2013 (b.). Error bars are $\pm\text{SE}$ ($n=4$).

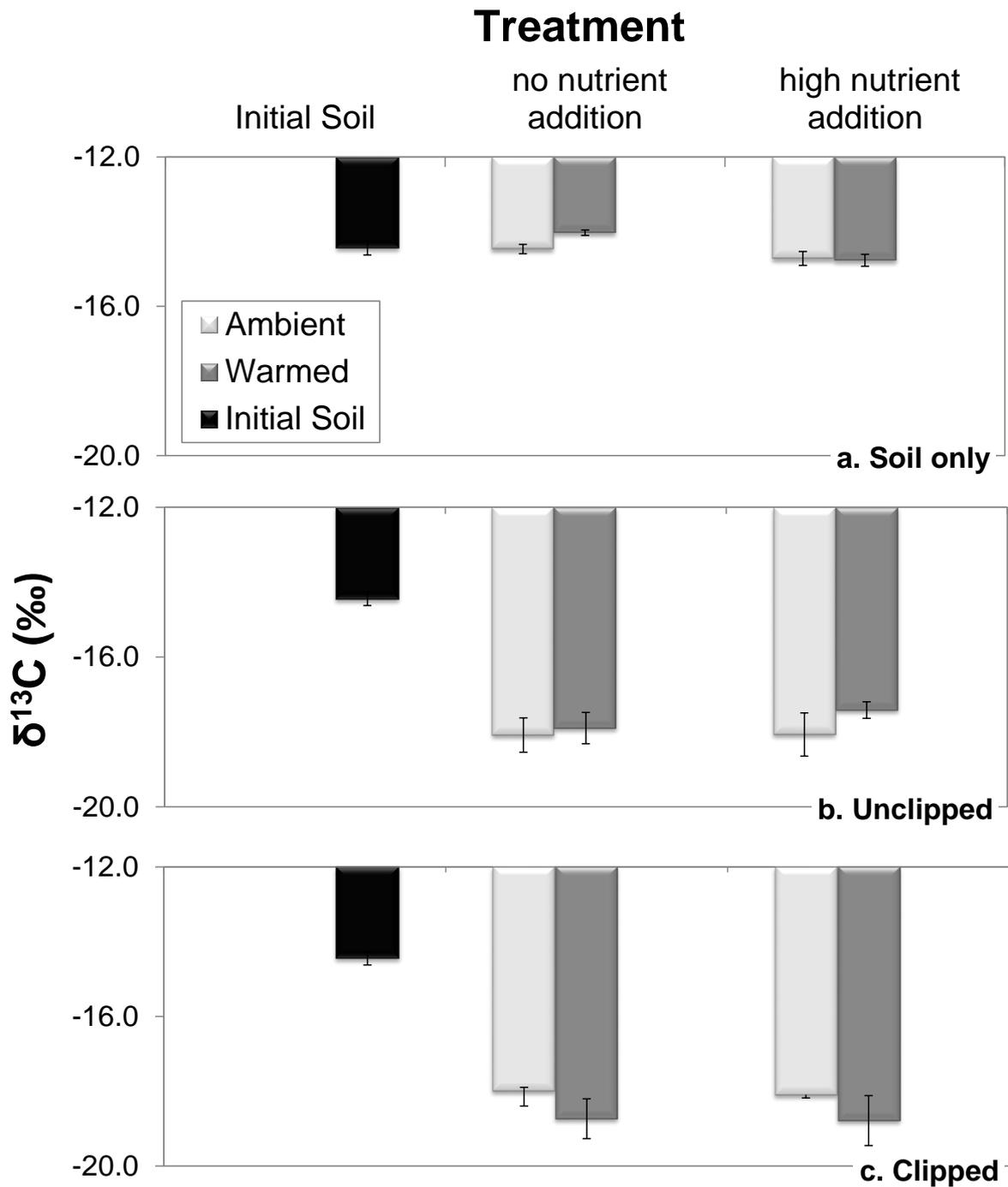


Figure 4.16. Soil sample results for the $\delta^{13}\text{C}$ analysis indicating the levels in the soil only (a.), clipped (b.) and unclipped (c.) planted lysimeters, 15 months after the initial soil was tested. Error bars are $\pm\text{SE}$ ($n=4$).

4.6.4. Total carbon content and carbon stocks

The carbon content data were used to calculate the soil carbon storage (Figure 4.17) and total carbon content of biomass dry weight (Figure 4.18) accumulated during the 15 months of experiments.

All ecosystems lost C, but there was some suggestion that the rate of C loss was lower in the high nutrient lysimeters. This appear to be the case even in the soil only lysimeters, suggesting that the impact of nutrient availability on decomposition rather than the impact of nutrient availability on new C inputs from plants may have been the key factor. No overall significant difference was detected between the warmed and control treatments.

The total carbon content of the above-ground biomass (Figure 4.18) was significantly greater ($P < 0.05$) in the lysimeters receiving high nutrient additions, but no warming effect was detectable for the high nutrient lysimeters. However, for the low nutrient lysimeters, the total carbon content per kg of dry weight was marginally higher in the warmed treatments of unclipped and clipped lysimeters.

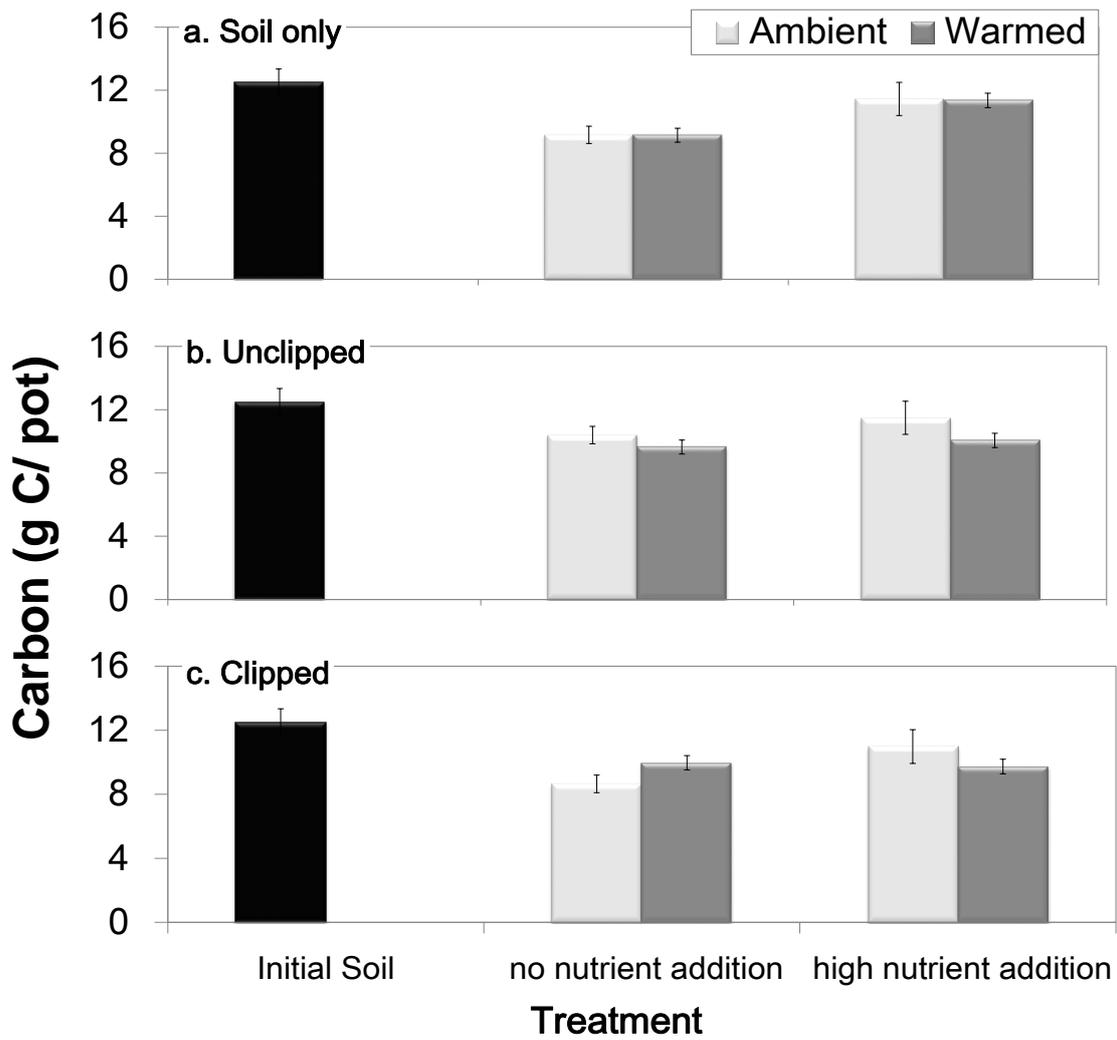


Figure 4.17. Soil carbon stock in the lysimeters after 15 months of experiments compared to the initial soil carbon stock. Error bars are \pm SE (n=4).

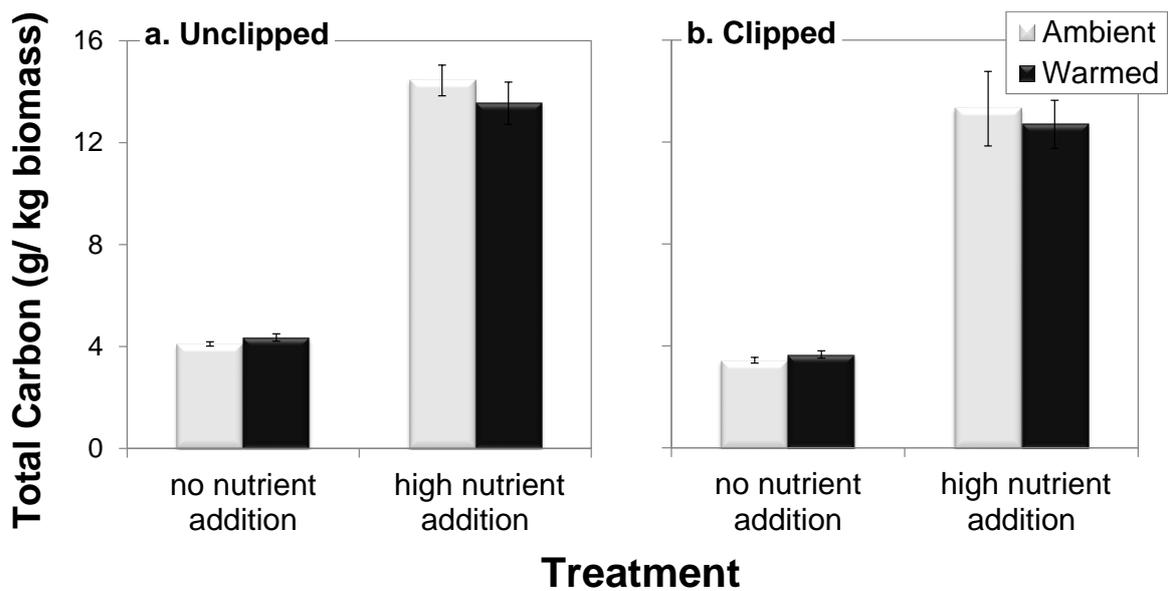


Figure 4.18. Total carbon stock of the above ground biomass at the end of the experiment - 15 months of growing. Error bars are \pm SE (n=4).

4.6.5. Rates of nutrients loss

The weekly leachate collected from all lysimeters was analysed for loss of nitrogen (N), phosphorus (P) and four main cations (magnesium – Mg^{2+} , calcium – Ca^{2+} , sodium – Na^+ and potassium – K^+) and the results presented as monthly averages. The change in the amount of nutrients loss over time was proportional to the amount of water leaked from each lysimeter according to the treatment applied. Due to the increase in demand from growing biomass and changes in air and soil temperatures the quantity of water added each week changed too.

4.6.5.1. Nitrogen (N) loss rates

Nitrogen loss results are illustrated in Figure 4.19 and just like all the other ecosystem parameters, the data was grouped in soil only lysimeters, unclipped planted lysimeters and clipped planted lysimeters. The data from the soil only lysimeters (AL, AH, WL and WH) indicate a significant difference between the low and high treatments once fertilisation started in August 2012 (Figure 4.19 – a). At the same time, no difference was observed between warmed and ambient once warming started in December 2012.

Results of the unclipped lysimeters (ALU, AHU, WLU and WHU) leachate analysis indicate a different trend to that observed in the soil only lysimeters. Starting with August 2012, when the fertilisation treatment first began, for every month until the end of the experiment a significant difference ($P < 0.016$) was visible between the four treatments (Figure 4.19 – b), except in February 2013 ($P = 0.066$) and June 2013 ($P = 0.214$). Contrary to the soil only lysimeters, the low nutrient treatments of the unclipped lysimeters recorded higher N losses compared to those receiving high nutrient levels. The constant higher levels lost by the ALU and WLU were also significantly different ($P < 0.05$) from each other once warming was applied and its effect established. A significant change also occurred after warming was applied in the high nutrient lysimeters, thus the warmed treatments lost more N in leachate than the ambient, reaching a maximum loss in March and April 2013, approaching the levels recorded in the low nutrient lysimeters.

The clipped lysimeters (ALC, AHC, WLC and WHC) analysis results show a similar trend to the unclipped, up to the point when the 1st clipping was applied in January 2013 (Figure 4.19 – c). A couple of months after that event the high nutrient lysimeters record a massive increase of N loss in leachate, almost five times the magnitude before clipping, but with a significant difference between ambient and warmed treatments only in February and July 2013 ($P < 0.03$). In the low nutrient addition lysimeters, after warming started, the data indicated slightly higher values for ALC although not statistically significant, except in December 2012 ($P < 0.013$).

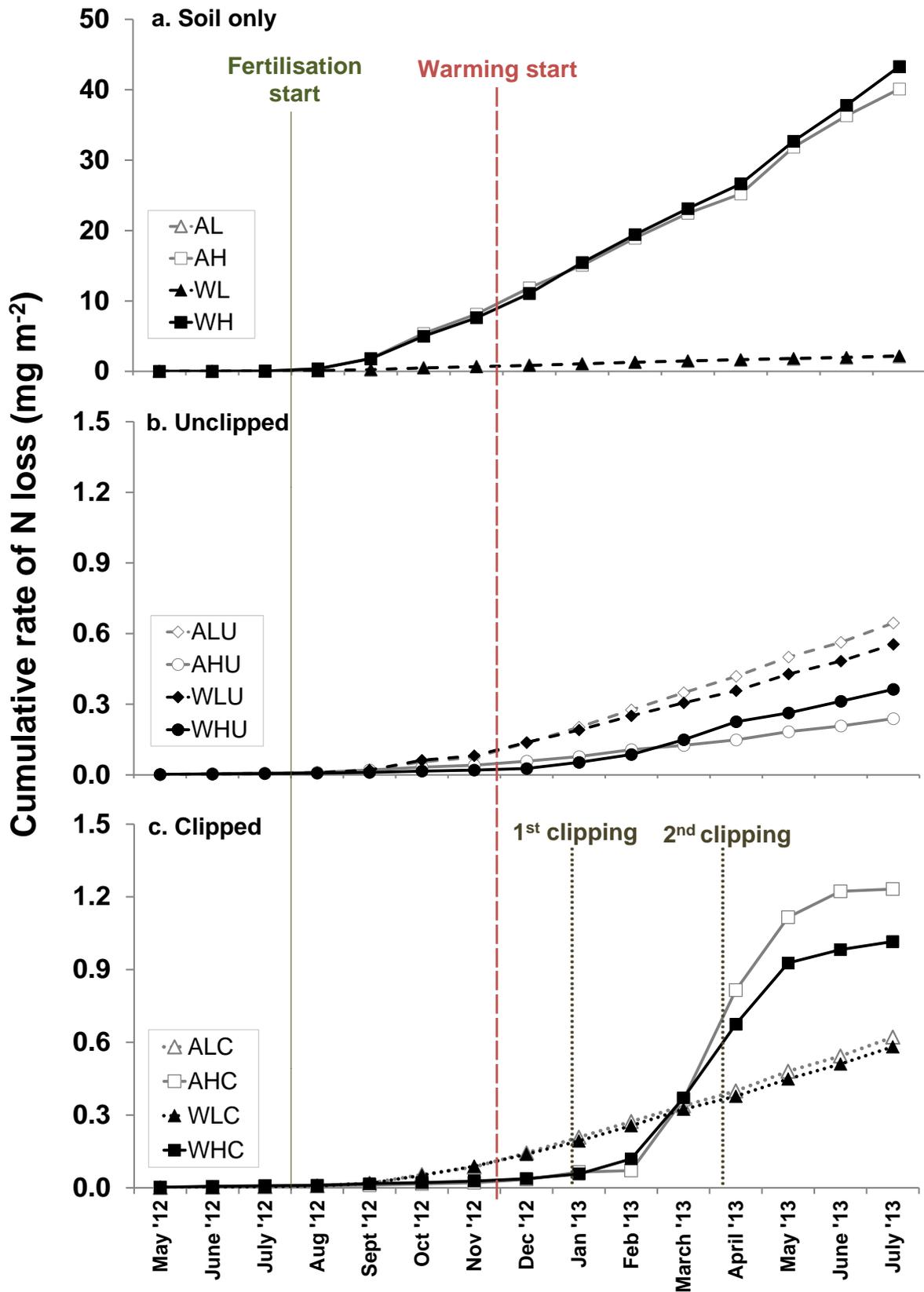


Figure 4.19. Cumulative rates of nitrogen loss in leachate, relative to the main treatments applied to the lysimeters: nutrient additions (continuous vertical line), warming (dashed vertical line) and clipping (dotted vertical line). Lysimeters were grouped by manipulations, as soil only lysimeters (a.), unclipped planted lysimeters (b.) and clipped planted lysimeters (c.). Error bars are \pm SE (n=4)

4.6.5.2. Phosphorus (*P*) loss rates

The phosphorus concentration in the leachate collected during the 15 months of the experiment is illustrated in Figure 4.20. The P loss data was presented in the three main groups: only lysimeters, unclipped planted lysimeters and clipped planted lysimeters. The key factor differentiating the treatments was the level of nutrient addition to the lysimeters, after August 2012 when fertilisation started. Further separation between treatments was recorded after December 2012 when the warming started, but only some of the lysimeters were significantly different from their ambient correspondent.

In the soil only lysimeters (AL, AH, WL and WH) the nutrient levels showed significant difference ($P < 0.001$) between added and no added fertiliser, from November 2012 until the end of the experiment in July 2013 (Figure 4.20 – a); the high nutrient lysimeters had greater P concentration in leachate. Once warming began, it made a significant difference ($P < 0.019$) only in the high nutrient lysimeters, with the ambient (AH) losing more P than the warmed (WH) lysimeters.

An opposite trend was visible for the unclipped planted lysimeters (ALU, AHU, WLU and WHU). Until February 2013 the low nutrient lysimeters lost more P than the high nutrient lysimeters. No differences were observed between treatments from March 2013 onwards. Warming had no effect on the amount of P released from the unclipped lysimeters.

The clipped planted lysimeters (ALC, AHC, WLC and WHC), behaved similarly to the unclipped ones (Figure 4.20 – c), presenting significant difference ($P < 0.049$) between high and low nutrient addition treatments up to and including February 2013. The low nutrient lysimeters lost more P than the high ones, until March 2013 when no more differences were shown. A small, but not significant difference was recorded in December 2012 between warm and ambient lysimeters within the low nutrient treatment, but the difference dropped was lost after the clipping began in January 2013.

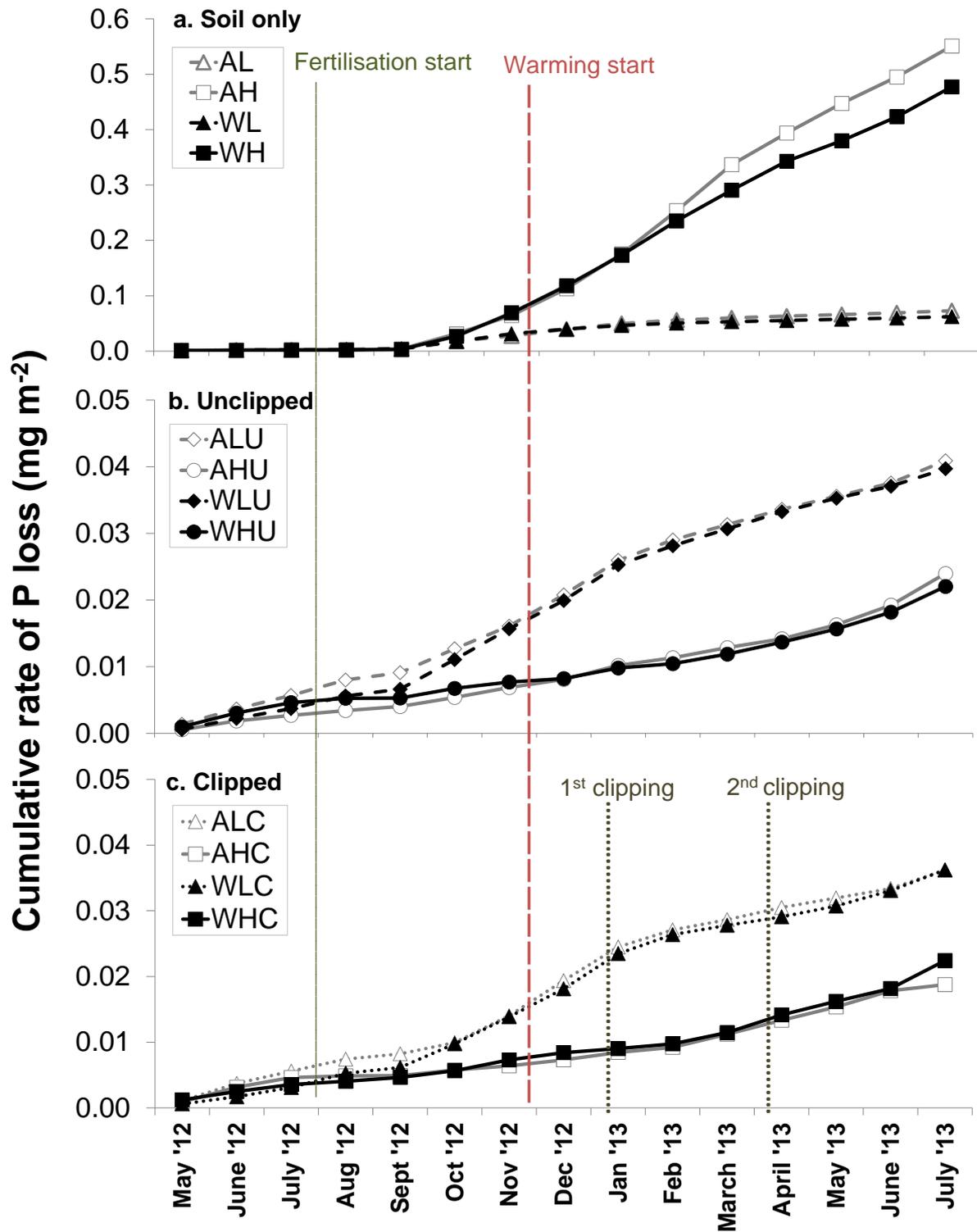


Figure 4.20. Cumulative rates of phosphorus loss in leachate, relative to the main treatments applied to the lysimeters: nutrient additions (continuous vertical line), warming (dashed vertical line) and clipping (dotted vertical line). Lysimeters were grouped by manipulations, as soil only lysimeters (a.), unclipped planted lysimeters (b.) and clipped planted lysimeters (c.). Error bars are \pm SE (n=4)

4.6.5.3. Cations loss rates

The results of the monthly leachate analysis indicated the trend of the four main cations loss: sodium – Na⁺, potassium – K⁺, calcium – Ca²⁺ and magnesium – Mg²⁺, during the 15 months of experiment. The data was grouped to illustrate the loss of these cations from soil only lysimeters (Figure C3-2), unclipped planted lysimeters (Figure C3-3) and clipped planted lysimeters (Figure C3-4).

For the soil only lysimeters (AL, AH, WL and WH), the levels of all cations lost began to significantly differentiate ($P < 0.001$) between low and high nutrient additions; greater loss from the high nutrient lysimeters (Figure C3-2). The nutrient effect on the cation loss rates was significant from the start of the fertilisation until the end of the experiment. Warming had no significant effect on the loss rates in leachate.

In the planted lysimeters, the Na loss trend was harder to separate between the treatments applied (a. – Figure C3-3 and C3-4). After warming started in the unclipped lysimeters (ALU, AHU, WLU and WHU), the only significant difference in Na loss rates was evident between the lysimeters receiving different nutrient levels. Later (February to April 2013) a significant difference was observed between warmed and ambient lysimeters with higher loss rates from the warmed treatments. In the clipped lysimeters, the Na loss rates were significantly higher for the lysimeters receiving fertiliser, in December 2012 and February to April 2013. Warming had only a marginal ($P > 0.075$) effect in April 2013, with higher loss rates in AHC compared to WHC.

The K loss from planted lysimeters (b – Figure C3-3 and C3-4) had higher rates when no fertiliser was added (significant in October 2012), but once warming started the loss rates were greater in the high nutrient lysimeters. In the unclipped lysimeters, the cation loss after warming was significantly higher ($P < 0.05$) in the high nutrient warmed lysimeters than ambient ones. For K⁺, Ca²⁺ and Mg²⁺, the concentration levels lost from the high nutrient clipped lysimeters were almost twice the magnitude of their unclipped equivalents, while the low nutrient lysimeters showed not to be affected by the 1st clipping session applied in January 2013.

4.7. DISCUSSION

4.7.1. Ecosystem carbon fluxes

The carbon fluxes recorded during 15 months of continuous experimental manipulation of a controlled grassland ecosystem have been analysed and partitioned between ecosystem respiration (ER) and gross primary production (GPP). The night time measurements began in October 2012 and represent the ER, indicating the amount of carbon released by the ecosystem to the atmosphere ($\mu\text{g C m}^{-2} \text{ s}^{-1}$). In parallel, GPP data illustrates the quantity of carbon taken up by the same ecosystem ($\mu\text{g C m}^{-2} \text{ s}^{-1}$).

4.7.1.1. *Effects of nutrient availability on carbon fluxes*

The first hypothesis assumed a reduction in carbon released from the soil with increased nutrient availability, with lower rates of release in the planted lysimeters especially when nutrient availability was low. The dominant effect of the nutrient addition seemed to be to directly reduce respiration rates rather than alter priming effects. This is evidence of the fact that soil C pools declined less rapidly in the high nutrient lysimeters in both the presence and absence of the plants. Results indicating that the net C uptake by the soil-plant system was an average $5.9 \mu\text{g C m}^{-2} \text{ s}^{-1}$ more in the high nutrient lysimeters than in their no additions correspondent. In contrast, the soil only lysimeters released $0.54 \mu\text{g C m}^{-2} \text{ s}^{-1}$ more from the high nutrient lysimeters, rejecting the formulated hypothesis, suggesting the importance of the soil-plant system interaction in controlling the C balance.

4.7.1.2. *Warming impacts on carbon fluxes*

The second hypothesis tested whether warming will enhance soil respiration and have greater magnitude in the absence of plants than in their presence. In the unclipped plots, in agreement with the hypothesis warming reduced net rates of C

uptake relative to the ambient treatment in the high nutrient lysimeters only (Figure 4.11). However, contrary to expectations this appeared to be caused more by a reduction in GPP in the warmed high nutrient lysimeters rather than an increase in ER (Figure 4.10 and 4.12). Therefore, while the hypothesis that net C release rates will be greater where nutrient availability is high was supported, the underlying mechanisms requires further study.

Melillo *et al.* (2002), found that despite the uncertainties in both estimates of carbon gains in the vegetation and measurements of carbon losses from soil due to warming, the estimated vegetation gains were at least as large as the measured soil losses. In addition, a number of other warming experiments in mid-latitude ecosystems, even without a dominant woody vegetation component, such as alpine meadows and grasslands have shown either small carbon losses or little change in carbon storage (de Valpine and Harte, 2001, Lin *et al.*, 2011). Warming may have its largest positive feedback effects in high-latitude ecosystems that contain small-stature or sparse woody vegetation and large pools of partially decomposed soil carbon that have accumulated under cold, wet conditions. If these soils undergo both warming and drying, they have the potential to lose large amounts of carbon as CO₂ to the atmosphere (Davidson *et al.*, 2000). This additional carbon release to the atmosphere can then result in more greenhouse effects, aggravating anthropogenic warming.

4.7.1.3. *Clipping influence over the soil carbon fluxes*

The third hypothesis tested if clipping will reduce C sequestration in plant biomass and thus reduce the strength of the link between nutrient and plant growth, by reduce nutrient immobilisation in plant biomass and adding readily decomposable organic matter to the soil. Thus it was expected that more C will be released due to warming in clipped lysimeters for both high and no nutrient additions. The hypothesis was not fully supported by the results, because clipping greatly reduced NEE in both ambient and warmed high nutrient lysimeters with the effect on ER in the ambient lysimeters being particularly strong. Further study is required to determine why ER increased so much in the high nutrient ambient lysimeters

but it could have been related to greater soil moisture in the ambient lysimeters promoting litter decomposition during this time (Figure 4.10)

By the time the second clipping was applied the biomass initially cut and added back to the lysimeters, did not fully decompose, thus adding more to the decomposing material around the edges of the lysimeters; sometimes growing mould due to constant humidity provided by the weekly water additions and poor ventilation caused by the existing standing biomass.

4.7.1.4. *Main and interactive effects of treatment on carbon fluxes*

The fourth hypothesis investigated whether at low nutrient concentrations, the gain in plant biomass will offset soil C losses. This assumption is slightly supported by marginally more plant biomass in the warmed lysimeters without added fertiliser and less for the high nutrient additions (Figure C3-5).

4.7.2. Changes in respired CO₂ source

Natural ¹³C abundance can help establishing the source of the carbon respired by the ecosystem with minimum of disturbance (Hanson *et al.*, 2000, Uchida *et al.*, 2010). The material used for this project consisted of C₄ soil as the source of older SOM and the C₃ plant as new SOM inputs. No significant differences were observed between treatments for the plant lysimeters and the low $\delta^{13}\text{C}$ ratios indicated that little of the CO₂ being released was derived from decomposition of the C₄ soil (Figure 4.15 - a).

4.7.3. Ecosystem properties and productivity

4.7.3.1. *Soil and vegetation physical and chemical characteristics (biomass yields, SOM, N, P)*

The increase in above-ground biomass in the presence of nutrient addition, can represent evidence of changes in C allocation within the ecosystem. On the other hand, Shaver *et al.* (1992) discovered that, in an arctic ecosystem, fertiliser addition initially increases nutrient concentrations in plant tissues, but later on, due to growth and carbon accumulation these high concentrations were diluted to the point where fertilised and unfertilised plants differ only in biomass. This apparently strong proportionality between biomass (and carbon) accumulation and nutrient accumulation bring evidence that carbon and nutrient cycles are tightly linked (Shaver *et al.*, 1992).

These results are supported by previous research suggesting that C-N coupling reduces the sensitivity of terrestrial carbon uptake compared to C alone, and a shift toward proportionally more carbon uptake in vegetation and less in soil organic matter (Joos *et al.*, 2001, Zhao and Running, 2010).

4.7.3.2. *Loss of nutrients from the ecosystem*

N and P losses were greater in the low nutrient lysimeters once fertilisation started due to increase of biomass in the high nutrient lysimeters and uptake. This may also have been related to the higher nutrient additions increasing plant growth and water use, thus leaking less water than their correspondent low nutrient treatments. However, even if the nutrient concentrations present in the low nutrient lysimeters was less than those of high nutrient lysimeters, the greater amount or leachate, increased the total level of N and P losses. Thus greater plant growth can reduce nutrient losses through if higher rates of evapotranspiration reduce leaching rates.

Therefore, the results indicated a further factor controlling the C stocks in the ecosystem. Because of the 5°C increase over the ambient conditions for some of the treatments, water became a secondary limiting factor in the warmed lysimeters. Analyses of the above-ground biomass, suggested that enhanced plant growth, and thus C accumulation, in the lysimeters receiving high nutrient additions, compared with those without, was limited by water availability. This can be explained by the higher water demand of the faster growing biomass in the high nutrient lysimeters, in addition to the increased evaporation due to warming. However, in the control lysimeters with added nutrients, the ecosystem benefited from the reduced nutrient limitation and used the water more efficiently at ambient temperature to grow more biomass.

4.7.3.3. *Changes in soil C stocks*

Despite the big changes in both ER and GPP, it is important to emphasise that there were few differences between the treatments in terms of changes in SOM stocks or its isotopic signature. This suggests that the losses of existing C and the formation of new soil C stocks was not controlled by climate or productivity, agreeing with the findings of Giardina *et al.* (2014). Therefore, the results indicate that intrinsic properties of the soil, perhaps related to its texture or mineralogy, may be more important, than previously recognised, in controlling ecosystem C storage. These suggestions requires further research and will be returned to in the next chapter. If observed more widely, then there are major implications for potential rates of C loss under warming, and C gains under elevated CO₂ (current loss and uptake rates may be over-estimated).

4.8. CONCLUSIONS

The total amount of C stored in soils is controlled by many factors, included but not limited to the type and productivity of vegetation, the presence of soil organisms, climatic conditions and nutrient availability. The interactive effect of some of these factors, investigated in this study suggests emphasises how complex the interactions between all these factors may be and emphasises the need for further multi-variable manipulation experiments to improve understanding.

Overall it was observed that although different levels of nutrient additions, temperature manipulation and clipping treatments were applied to the ecosystem under investigation, the carbon stocks in soils did not suffer considerable changes.

CHAPTER V

FIELD AND LABORATORY EVIDENCE OF NUTRIENT ENRICHMENT AND TEMPERATURE CONTROLS OVER CARBON STORAGE

5.1. INTRODUCTION

This final chapter aims to bring together all the knowledge gathered from the experiments carried out in this project, and are described in Chapters II, III and IV. The overarching aim of the entire project was to assess how interactions between C and nutrient cycling control C storage in terrestrial ecosystems, and their responses to global change. I hypothesised that the links between decomposition and plant growth would be the key to determining the response of the soil C stocks to N deposition and climate change. Thus, many of the key processes in the terrestrial C and N cycling were investigated under different field and laboratory conditions.

Chapter II presented the results of a full-factorial experiment, investigating the response of soil C fluxes and net ecosystem C exchange to inorganic additions of nutrients N and P. This study took place at Wardlow Hay Cop, in the Peak District (PD), an area known to be heavily affected by high rates atmospheric N deposition. The main findings revealed that long-term experimental nutrient manipulations (over 20 years), have the potential to reduce decomposition rates, with no corresponding reduction in productivity. These results demonstrate that, by reducing the strength of the link between decomposition and plant productivity (plant productivity was less dependent on N release from decomposition), N deposition has the potential to increase C storage in semi-natural acidic grasslands.

Chapter III described the changes in soil C fluxes and litter decomposition rates in intensely managed grassland plots (treatment applied approximately 5 years), in the presence or absence of N-fixing legumes. These investigations took place on the WEB site at North Wyke Research Centre. Measurements of soil C fluxes were compared between two types of sward, one containing a mixture of grass-legume-forb and the other, representing the control, with grass only. Contrary to the results in Peak District, the soil C fluxes and woody litter decomposition rates (but not grass decomposition) increased in the presence of legumes, rejecting one of the formulated hypotheses. However, ecosystem productivity increased in the plots containing legumes and forbs.

Chapter IV investigated a grass ecosystem under controlled laboratory conditions, where both nutrient availability and soil temperature were manipulated. The aim of this chapter was to assess the response of Net ecosystem productivity (NEP), Gross primary productivity (GPP) and Ecosystem respiration (ER) to changes in nutrient availability and temperature. Contrary to expectations, despite big differences in the productivity, with the nutrient treatments and warming increasing rates of decomposition, there was very little effect on soil C storage. There is a growing body of literature suggesting that soil C storage may be controlled more by intrinsic soil properties (chemical and physical protection) than by climate or plant productivity. The results seem to support this suggestion.

Based on the findings of the above chapters, Figure 5.1 & Figure 5.2 illustrate the amended Conceptual Model, representing the C and nutrient cycle interactions, and incorporates the increase or decrease of some of the measured/estimated processes, in the field and the laboratory scale experiments. The question marks emphasise that the ultimate effect on soil C storage is harder to predict, given that big differences in decomposition rates and plant productivity had little effect on soil C storage in the experiment presented in Chapter IV. These are the areas that could benefit greatly, in the future, from further experiments and empirical data. This chapter gathers the results of all the above experiments and emphasises how this PhD has improved the understanding of C storage controls and its potential responses to future climate change.

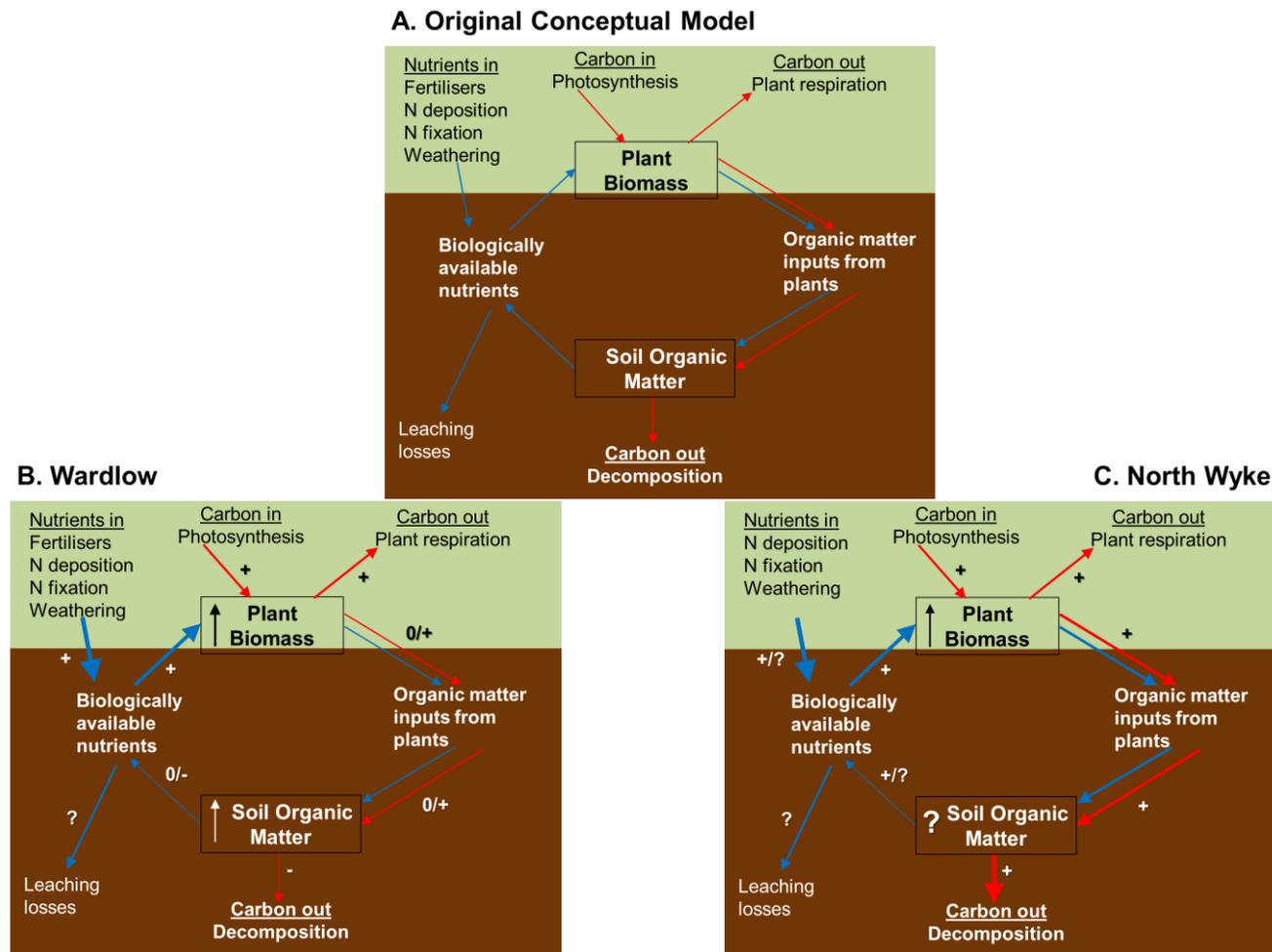


Figure 5.1. Original (A) and modified conceptual models (B, C), illustrating the effects of the inorganic nutrient addition in Wardlow (B) and potential increased rate of N fixation at North Wyke (C). The weighting and labelling of the arrows indicates the effects on the key processes (+ = increase, - = decrease, 0 = no change, and ? = unclear). In Wardlow, the productivity of the ecosystem was maintained or increased despite the rate of decomposition declining. This is reflected in the thinner arrow for the decomposition flux, but the maintained or increased inputs from plant biomass likely increased soil organic matter. For North Wyke, the effects on soil organic matter are less clear because both C inputs and rates of decomposition increased.

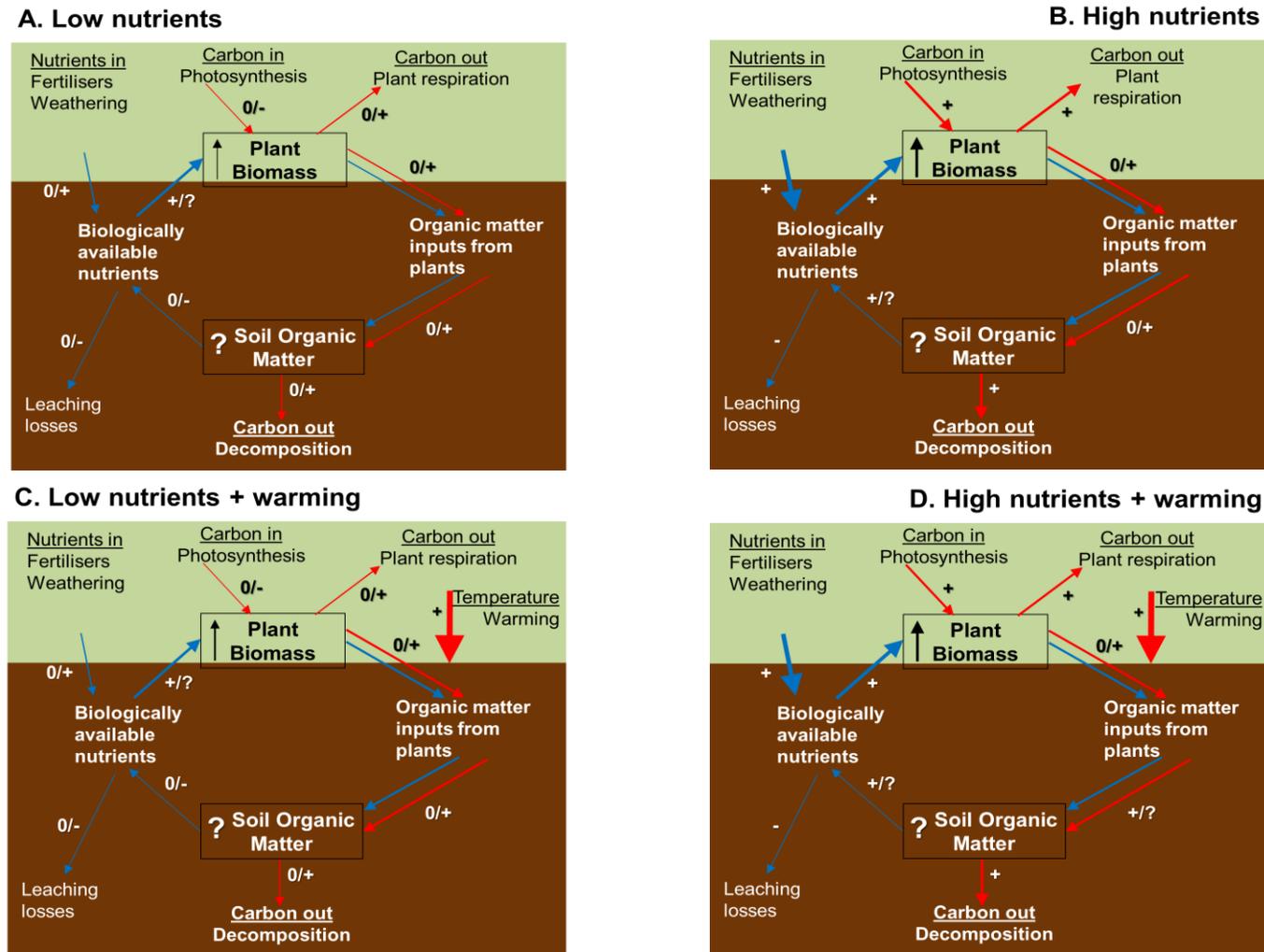


Figure 5.2. Conceptual models showing the effects of the nutrient and temperature manipulations carried out in the laboratory. Low nutrient (A and B) and high nutrient treatments (C and D), and ambient (A and C) and warmed (B and D) treatments are shown. Both nutrient addition and warming tended to increase the rates of the key processes (decomposition and net primary productivity), but it was much less clear how this affected net ecosystem exchange and soil carbon storage, emphasising the need for further research.

5.2. NUTRIENT AVAILABILITY AND DECOMPOSITION RATES

Litter decomposition rates and soil C efflux measurements were used to determine the effect of nutrient availability on potential rates of C release from soils. Field experiments setup in Wardlow, Peak District and North Wyke, Devon were designed to assess different mechanisms responsible for the release of C from soils.

Based on the similar design of the two experiments, the data can be compared and used to test the following hypothesis:

- As nutrient availability increases, decomposition rates will decrease because of direct effect of N suppressing in decomposition and due to a potential reduction in the priming effect; plants may release less labile C into the rhizosphere when nutrient availability is high reducing the activity of microbes responsible for decomposition.

Contrasting results were obtained from the two experiments, which support the hypotheses formulated in Wardlow but reject the hypothesis for North Wyke data. These responses may be explained by the dissimilarities in the two experiments, both designed to investigate different processes in the C-N interaction cycle. The experiments investigated the soil C fluxes, productivity and decomposition rates, but in different types of grasslands, and in response to different sources of nutrient availability. At Wardlow, the responses were assessed in relation to mineral N additions (background N deposition and inorganic N and P additions) while at North Wyke, biological N fixation by legumes was the source of potential N enrichment in the grassland, and plant diversity changed very strongly at the same time.

Both inorganic nutrient additions in the semi-natural grassland at Wardlow and biologically fixed N in the managed mixed-sward grassland at North Wyke were compared in order to bring further understanding to the interactions between terrestrial C and N cycles. The parameters measured were assessed in relation to increased nutrient availability and by quantifying the subsequent changes in productivity and biodiversity.

5.2.1. Reasons for difference between Wardlow and North Wyke

While the N addition reduced decomposition in the acidic grassland at Wardlow, the presence of legume, and/or forbs, increased rates of litter decomposition in the grass-legume-forbs plots at North Wyke. In the latter study, the greater productivity, or the greater plant diversity and potentially greater diversity of the associated decomposer community, could have promoted the decomposition of the novel substrate (woody litter bags).

Other reasons for the differences observed in the results of the two experiments might be the age of the experimental setup, the size of the plots investigated and the management practices. The plots at Wardlow were established for approximately 20 years when the experiments described in Chapter II began, while those at North Wyke were running for only five years. At the same time, at Wardlow the soil in the plots was generally undisturbed, while at North Wyke, as part of the management, the plots were first ploughed before sowing the grass seed mixtures. In the soil, the equilibrium between C inflows and outflows can be disturbed by such management practices, and it requires different periods of time before it reaches a new equilibrium (Guo and Gifford, 2002). It is known that during this process the soil might act either as a C source or sink, and that is what might have set apart the results obtained from the two experimental setups. It is plausible that at North Wyke the full equilibrium was not reached when the measurements were taken, thus the results recorded the soil C fluxes associated with the soil preparation that disturbed the soil structure and broke the aggregates. The disturbance could have affected soil structure or the structure of the microbial community resulting in the more rapid release of more readily C to the atmosphere.

Another factor contributing to the difference in results between Wardlow and North Wyke experiments could be the difference in nutrient addition type and background N deposition rates. While Wardlow is situated in an area recording over $9.4 \text{ kg N h}^{-1}\text{y}^{-1}$ of total oxidised N deposition, the plots at North Wyke are exposed to levels between 7.7 and $9.4 \text{ kg N h}^{-1}\text{y}^{-1}$ (Galloway *et al.*, 2004,

Goulding *et al.*, 1998). At the same time, the inorganic N additions at Wardlow and the biological N fixation at North Wyke might have affected differently the soil processes responsible for controlling the C fluxes and decomposition rates. Although it could not be proven that N fixation did occur, the results obtained might have been influenced by the background N deposition changing the soil pH and interfering with the legumes capacity to fix N more efficiently. However, the mineral N concentrations in the legume-grass-forb plots tended to be higher suggesting there was some increase in N fixation rates.

The contrasting observations, could have been result of spatial variability of the sampling, related to the different size of the plots investigated at both sites. The plots at Wardlow had an area of approximately 9 m², while those at North Wyke covered approximately 450 m². Although, by having additional internal replicate sampling locations in the bigger plots at North Wyke, it is possible that this was not enough to deal with the special variability, as suggested by studies carried out on the same farm platform (Peukert *et al.*, 2012).

Other important factors that could explain these differences in results, are the plant diversity and the associated soil microbial community controlling the decomposition rates. The soil microbial community can be driven by changes in pH, soil texture and soil nutrient availability (Lauber *et al.*, 2008), and thus, they can become specialised for certain types of ecosystems and adapted to a specific type of litter input. There is further evidence that the microbial activity, as the main driver of decomposition, is bound by local-scale controls (Bradford *et al.*, 2014), such as temperature, precipitation, C and nutrient input types.

The results from North Wyke in chapter III are therefore more challenging to interpret in the context of changes in links between C and nutrient cycling, as the change in plant and/or microbial biodiversity may have been more important than the potential change in N availability if N fixation did increase.

5.2.2. Explanations for Wardlow findings: the direct effect of nutrient availability on decomposition

In the Wardlow experiment, the mineral N additions reduced the decomposition rates, but stimulated an increase in plant productivity, as illustrated in Figure 5.1. Factorial additions of fertilizer containing N, with or without P, stimulated plant growth, but reduced decomposition in the acidic grassland (Phoenix *et al.*, 2012). It has been suggested that N addition can have the greatest direct negative effect on the decomposition of low quality organic matter (Knorr *et al.* 2005). The soils of acidic grassland have organic matter rich, with a 7 cm thick organic horizon. While it is unlikely that the decomposition of fresh litter was inhibited by N addition, the rate of decomposition of older more decomposed organic matter within the soil may have been reduced.

The potential for decomposition rates to decline despite productivity increasing in response to N addition also been observed in other studies. Working on Hawaiian mountain forest and rainforests respectively (Hobbie and Vitousek, 2000, Torn *et al.*, 2005) found that even when N is the limiting nutrient, fertiliser additions can decrease slightly the rate of decomposition, while productivity can still be stimulated by the newly available N. In these studies, it was also suggested, that the low quality of the litter inputs may result in N addition directly inhibiting decomposition, while the slow rates of decomposition mean that microbial growth rates are very slow and therefore the microbial community may not be as limited by N availability as expected (Hobbie and Vitousek, 2000).

5.2.3. Explanations for Wardlow findings: The priming effect control over decomposition rates

Alternatively, decomposition rates may decline as a result of N addition, not because of a direct effect of N on decomposition rates, but rather because of reduced C inputs from plants decreasing labile C supply to the decomposers (Janssens *et al.*, 2010). This in turn could result in a decline in priming effects, with the reduction in labile C release to the soil, decreasing the activity of microbes responsible for decomposition (Kuzyakov, 2002).

Finally, the Wardlow experiment also studied the calcareous grassland, where P additions had a greater effect on productivity and plant diversity than the N additions. This would have provided the ideal opportunity for understanding the impacts of N addition on a non-N-limited ecosystem and therefore it is unfortunate that the site was too exposed to the wind to allow good quality CO₂ flux measurements to be made.

5.3. TEMPERATURE AND NUTRIENT INTERACTIONS CONTROLLING SOIL CARBON STORAGE

Changes in carbon storage in terrestrial ecosystems can affect rates of climate change. The focus of the experiment carried out in Chapter IV was on how nutrient availability affects ecosystem responses to changes in temperature.

Understanding how temperature changes affect soil C storage in contrasting ecosystems, is key to predicting the magnitude of future climate change, due to the feedback potential between terrestrial ecosystems and climate (IPCC, 2013). Thus, measurements of soil carbon fluxes under different temperatures and nutrient treatments, during the day and night, were designed to elucidate some of the controls on the soil storage. At the same time, the three experiments can be used to test the accuracy of the results, by comparing the data from the field with soil C fluxes responses obtained under treatment manipulations in laboratory conditions, but not when warming was applied. The information obtained from the two field experiments on nutrient availability control over soil C fluxes, decomposition and ecosystem productivity (Figure 5.1) was complemented by the temperature manipulations data collected in the lab (Figure 5.2).

The results obtained from the two field experiments contradicted each other; however, as explained in section 5.2.1, different local conditions might have been the cause. The nutrient manipulations in the controlled laboratory conditions, showed that high N additions increased the C fluxes and the ecosystem productivity. In addition to this, the warming had very little effect on the C fluxes, the main controlling factor appearing to be the level of nutrient added to the lysimeters. Although, there could be other mechanisms in addition to nutrient levels, controlling the response of these grassland ecosystem to warming and thus the decomposition rates recorded.

Soil organic matter is very heterogeneous and there are lots of mechanisms in soils that can protect this organic matter within the soil matrix. The turnover rate of the different SOM compounds varies due to the complex interactions between biological, chemical, and physical processes in soil (Post and Kwon, 2000). Many organic compounds in the soil are intimately associated with mineral soil particles.

The existence of these specific stabilising mechanisms make it difficult to predict the behaviour of SOM decomposition to warming (Davidson and Janssens, 2006). Working out what the feedback effect this warming could have for climate change is particularly difficult, mainly because the diverse soil organic compounds exhibit a wide range of kinetic properties, which determine the intrinsic temperature sensitivity of their decomposition (Davidson and Janssens, 2006). The fact that warming and nutrient manipulations had big effects on productivity and rates of decomposition but did not have major effects on soil C stocks, suggests that the importance of these protection mechanisms may currently be underestimated.

5.4. FUTURE CARBON SEQUESTRATION IN SOILS

The results obtained in chapter IV, indicates that nutrient addition increased photosynthetic rates by approximately 45 %, compared to the lysimeters under ambient conditions. At the same time, the significant increase in above ground biomass in the lysimeters receiving high levels of fertiliser did not correspond to a similar trend in below ground C concentrations.

However, the lack of significant increase in the soil C storage could be explained by factor previously not considered in this project. Thus, the experiment suggests that while there will not be big losses due to warming (see section 5.3), any process that increases plant productivity C input into soils, such as elevated CO₂, may not increase soil C stocks unless the organic matter becomes protected in the soil.

Recently, there have been further investigations into this matter and results indicate that soil mineralogy could be responsible for the lack of significant response in soil C stocks to temperature (Giardina *et al.*, 2014, Hartley, 2014). Previous studies have emphasised that the soil C stocks are mainly controlled by the chemistry of the soil organic matter itself, but there is evidence that the processes within the soil and the soil composition itself has a key role to play (Schmidt *et al.*, 2011, Torn *et al.*, 2005).

5.5. LIMITATIONS

The main limitation of this project was time and weather. Although a lot of preparation went into the design and timing of each experiment, there were factors that could not be fully controlled. Additional trips to make measurements both in Peak District and North Wyke, would have increased the confidence in the data obtained. The summer of 2012 was anomalously wet. A drier summer during the work carried out for Chapter III could have increased the chances of making additional measurements of soil CO₂ fluxes and net ecosystem carbon exchange, making it more easily comparable to the PD study. Both PD and NW studies were carried towards the end of broader original experiments, thus limiting the time during which these extra measurements could be taken.

The 'Pots' experiment benefited from the longest running duration (15 months) and being constructed from scratch, created the opportunity to control and monitor all the manipulations necessary to test the formulated hypotheses. Even so, technical issues with equipment, planned or unplanned power cuts in the building and human error, were factors that could not always be predicted and dealt with in time, but efforts were made to diminish their effect on the smooth running and accuracy of the data obtained. Considering the duration of this project, all the efforts were made to maximize the time and resources available in order to successfully complete the tasks set beforehand, necessary to meet the aims and objectives of this project.

Future research:

Some key issues have been identified throughout this project, and although more understanding was brought to the factors controlling the soil C responses to climate change, there are still areas that could benefit from further research. A key study, that could further the understanding of the soil processes responsible for the maximising the C sequestration in soils, could focus on the edaphic factors controlling the soil C fluxes responses to warming. This will also benefit from directly examining a range of soil types to better predict the effects of warming on C release.

5.6. CONCLUSIONS

In this PhD, I have demonstrated that inorganic N addition can reduce decomposition rates, without reducing of productivity, therefore increasing the potential for C sequestration. This result also demonstrates that that nutrient availability controls the strength of the link between plant productivity and the rate of decomposition in semi-natural grasslands. On the other hand the impacts of increasing rates of N fixation by legumes on rates of decomposition requires further study, since the increase in productivity and decomposition rates under legumes may have been caused by changes in plant and microbial diversity rather than N availability *per se*.

Finally, although nutrient availability played a key role in controlling flux rates (primary productivity and ecosystem respiration) and their response to warming, C stocks and $\delta^{13}\text{C}$ analyses showed that over a year's worth of warming and nutrient manipulations made little difference to the amount of soil C stored, indicating that intrinsic edaphic factors may have greater role in controlling C storage than temperature and rates of C input. This important finding requires further study regarding its broader applicability.

APPENDICES

FIGURES, PICTURES, TABLES AND LABORATORY METHODS

Appendix A

Chapter II

A1. Figures

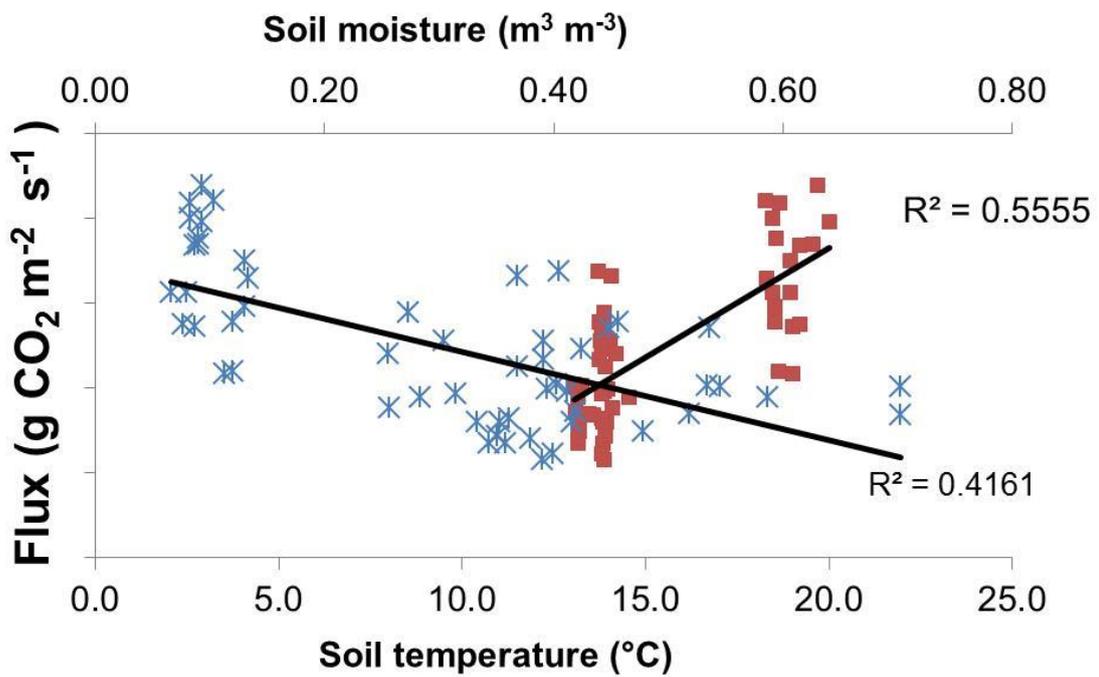


Figure A2-1. Soil temperature vs. moisture in the acidic grassland

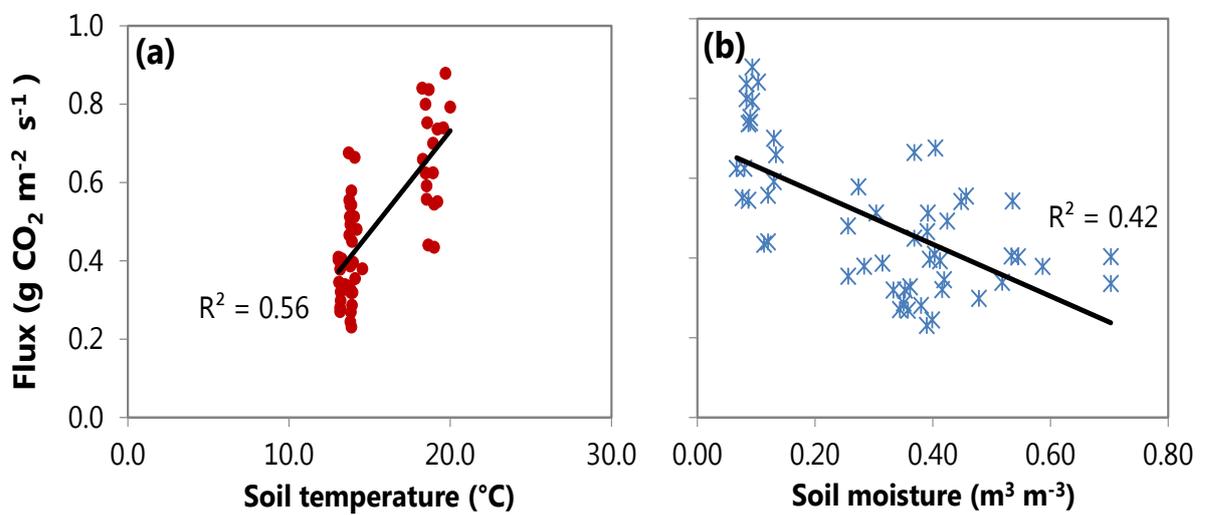


Figure A2-2. Correlations between soil CO₂ flux and temperature and between soil CO₂ flux and moisture, in the acidic grassland

Appendices

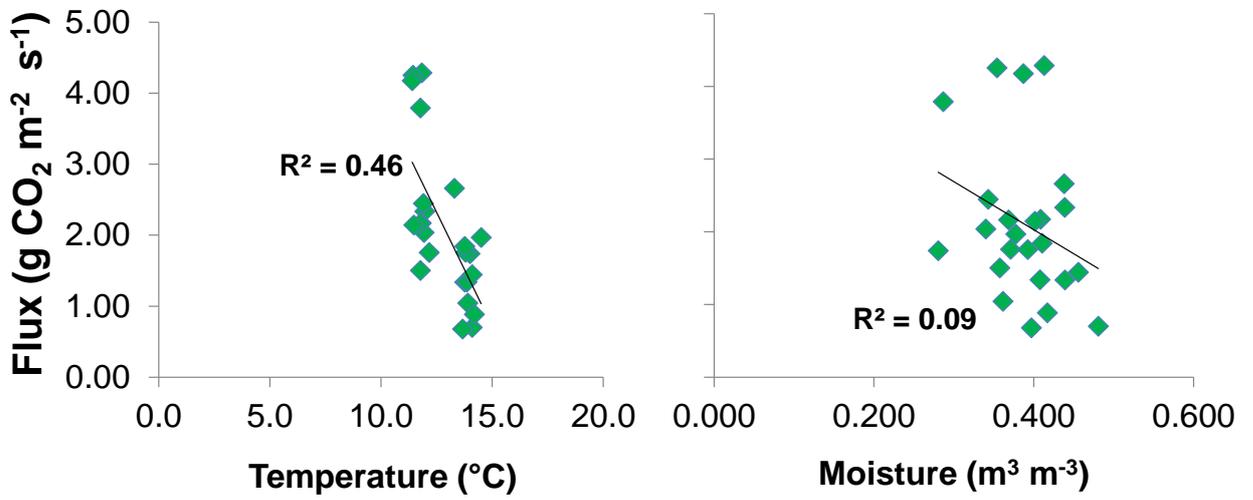


Figure A2-3. Correlations between soil CO₂ flux and temperature and between soil CO₂ flux and moisture, in the calcareous grassland, excluding the first measurement session in May, due to interference from initial disturbance.

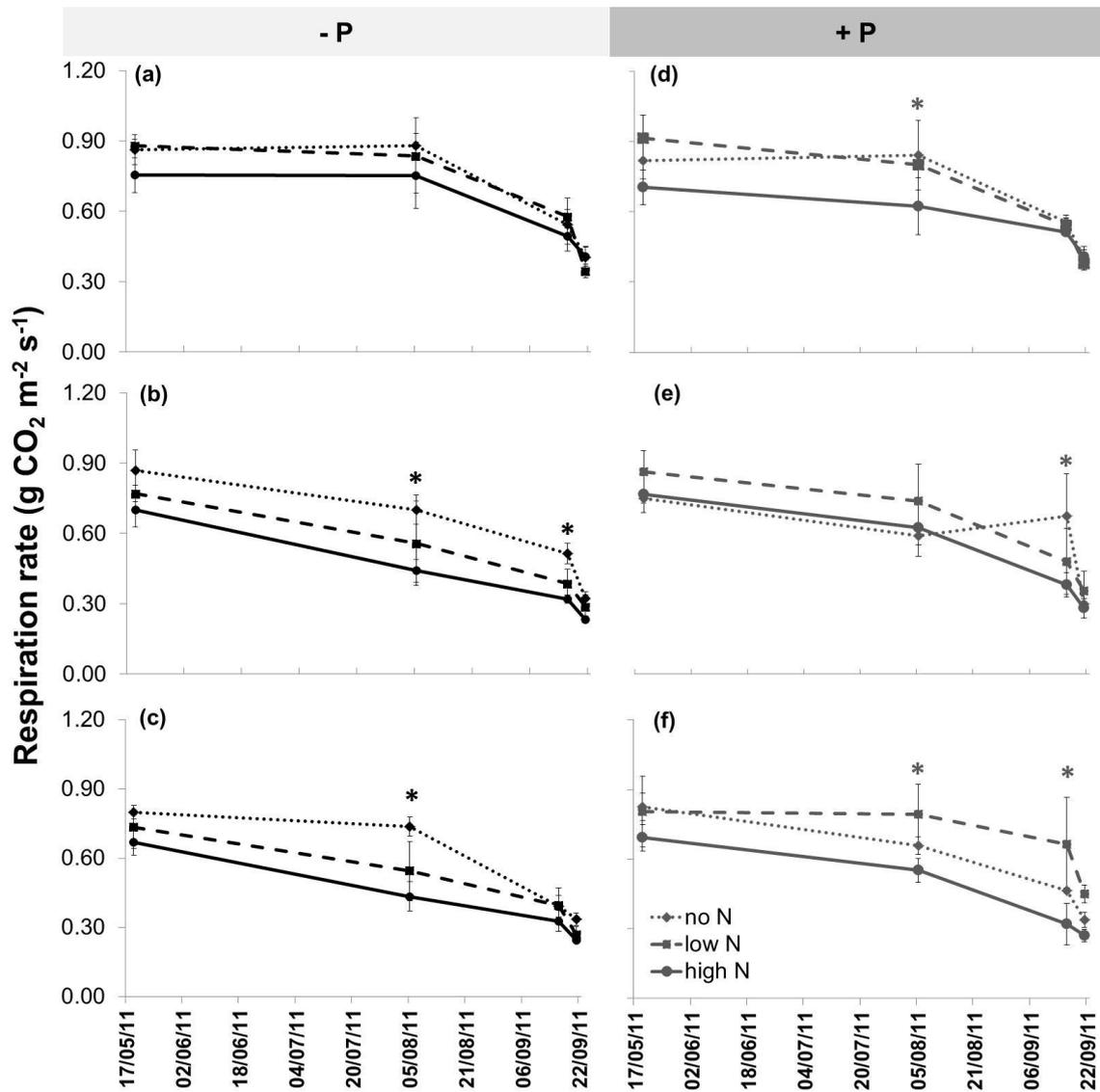


Figure A2-4. Seasonal fluxes recorded in the acidic grassland at *RMS* (a, d), *MS* (b, e) and *S* (c, f) collars, for the plots without (a, b, c) and those with (d, e, f) previously added P. Significant differences between treatments are indicated by '*'. Error bars are ±1SE (n=3).

Appendices

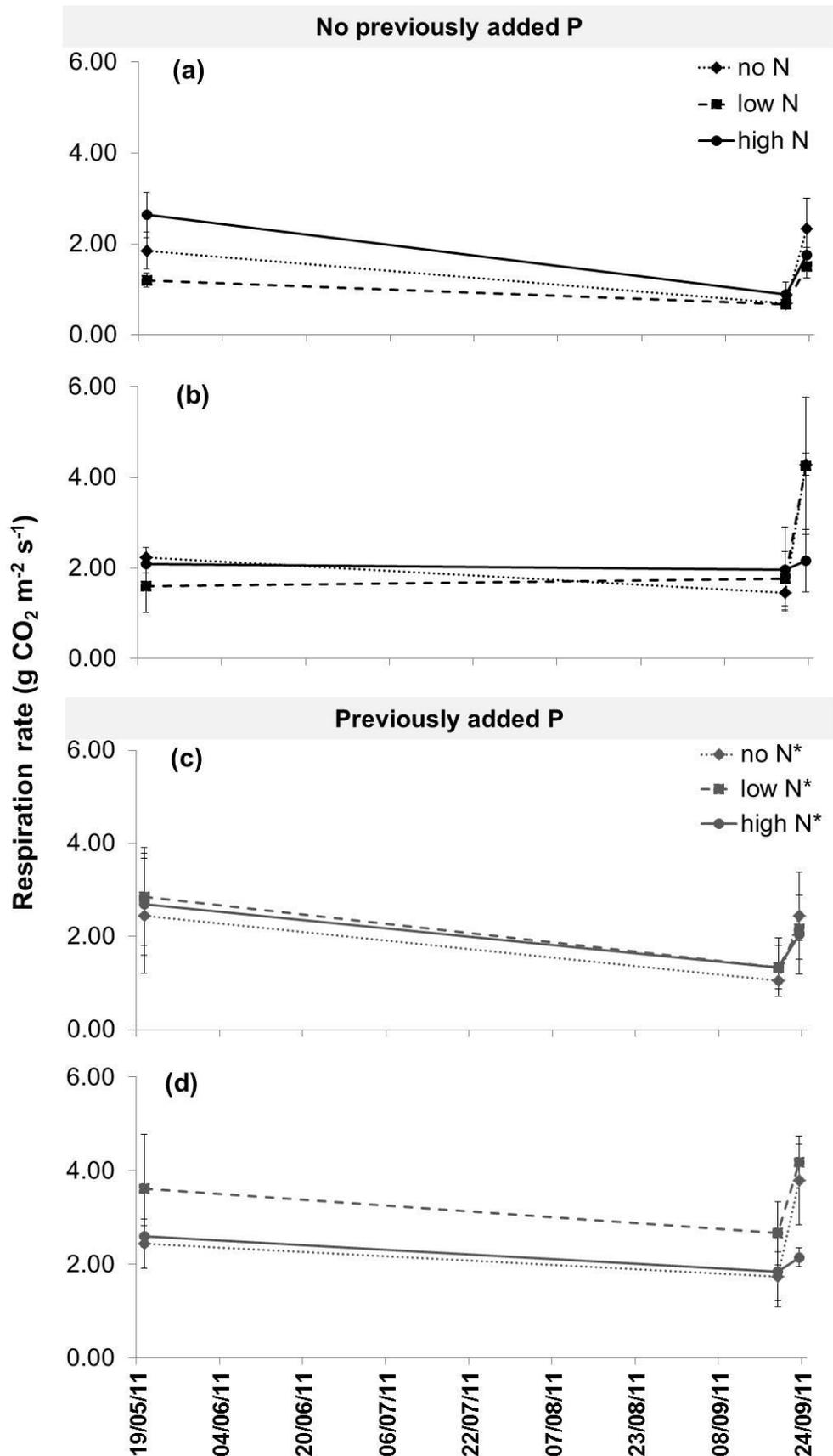


Figure A2-5. Seasonal fluxes recorded in the calcareous grassland at *RMS* (a, c) and *S* (b, d) collars, for the plots without (black lines) and those with (grey lines) previously added P treatment. Error bars are ± 1 SE (n=3).

Appendix B

Chapter III

B1. Tables

B2. Figures

B3. Pictures

B4. North Wyke Standard Laboratory Methods

Appendices

B1. Tables:

Table B1-1. Detailed content of the three seed mixture main treatments applied to the WEB experiment plots in 2008

Seed rate kg ha ⁻¹	%	Grass mix	Seed rate kg ha ⁻¹	%	Grass + Legume mix	Seed rate kg ha ⁻¹	%	Grass + Legume + Forb mix
6.50	21.67	certified Aberstar perennial ryegrass	4.88	21.67	certified Aberstar perennial ryegrass	3.90	21.67	certified Aberstar perennial ryegrass
6.50	21.67	certified Orion tetraploid perennial ryegrass	4.88	21.67	certified Orion tetraploid perennial ryegrass	3.90	21.67	certified Orion tetraploid perennial ryegrass
6.50	21.67	certified Sparta cocksfoot	4.88	21.67	certified Sparta cocksfoot	3.90	21.67	certified Sparta cocksfoot
5.00	16.67	certified Promesse timothy	3.75	16.67	certified Promesse timothy	3.00	16.67	certified Promesse timothy
5.00	16.67	certified Cosmolit meadow fescue	3.75	16.67	certified Cosmolit meadow fescue	3.00	16.67	certified Cosmolit meadow fescue
0.50	1.67	commercial meadow foxtail	0.38	1.67	commercial meadow foxtail	0.30	1.67	commercial meadow foxtail
30.00	100.00		22.50	100.00		18.00	100.00	
			0.60	5.22	certified Altaswede red clover	0.60	5.22	certified Altaswede red clover
			0.50	4.35	certified Milvus red clover	0.50	4.35	certified Milvus red clover
			0.50	4.35	native Essex red clover	0.50	4.35	native Essex red clover
			0.40	3.48	certified S184 wild white clover	0.40	3.48	certified S184 wild white clover
			1.00	8.70	certified Dawn alsike clover	1.00	8.70	certified Dawn alsike clover
			1.00	8.70	certified Giada birdsfoot trefoil	1.00	8.70	certified Giada birdsfoot trefoil
			4.50	39.13	commercial Sainfoin	4.50	39.13	commercial Sainfoin
			2.00	17.39	commercial sweet clover	2.00	17.39	commercial sweet clover
			1.00	8.70	certified Virgo Pajbjerg yellow trefoil	1.00	8.70	certified Virgo Pajbjerg yellow trefoil
			11.50	100.00		11.50	100.00	
						1.00	25.00	certified Puna chicory
						2.00	50.00	Burnet forage herb
						0.40	10.00	Yarrow forage herb
						0.30	7.50	Lesser Knapweed (Centaurea nigra)
						0.20	5.00	Ox-eye Daisy (Leucanthemum vulgare)
						0.10	2.50	Common Sorrel (Rumex acetosa)
						4.00	100.00	
30.00	Total		34.00	Total		33.50	Total	

Table B1-2. Detailed soil moisture, temperature and fluxes recorded during the two sampling sessions

			Soil temperature (°C)			Soil moisture (%)		Flux (g CO ₂ m ⁻² h)
Session	Collar	Treatment	2 cm	5 cm	8 cm	2 cm	5 cm	
September '12	RMS	G	17.18	16.17	15.73	0.117	0.506	0.61
	RMS	GLF	17.22	16.34	15.90	0.114	0.442	0.65
	S	G	17.53	16.42	15.89	0.137	0.504	0.65
	S	GLF	17.68	16.60	16.10	0.126	0.492	0.68
March '13	RMS	G	5.88	5.48	5.18	0.233	0.654	0.61
	RMS	GLF	5.50	5.12	4.83	0.235	0.603	0.65
	S	G	6.20	5.66	5.33	0.216	0.650	0.65
	S	GLF	6.02	5.65	5.36	0.241	0.652	0.68

Appendices

B2. Figures:

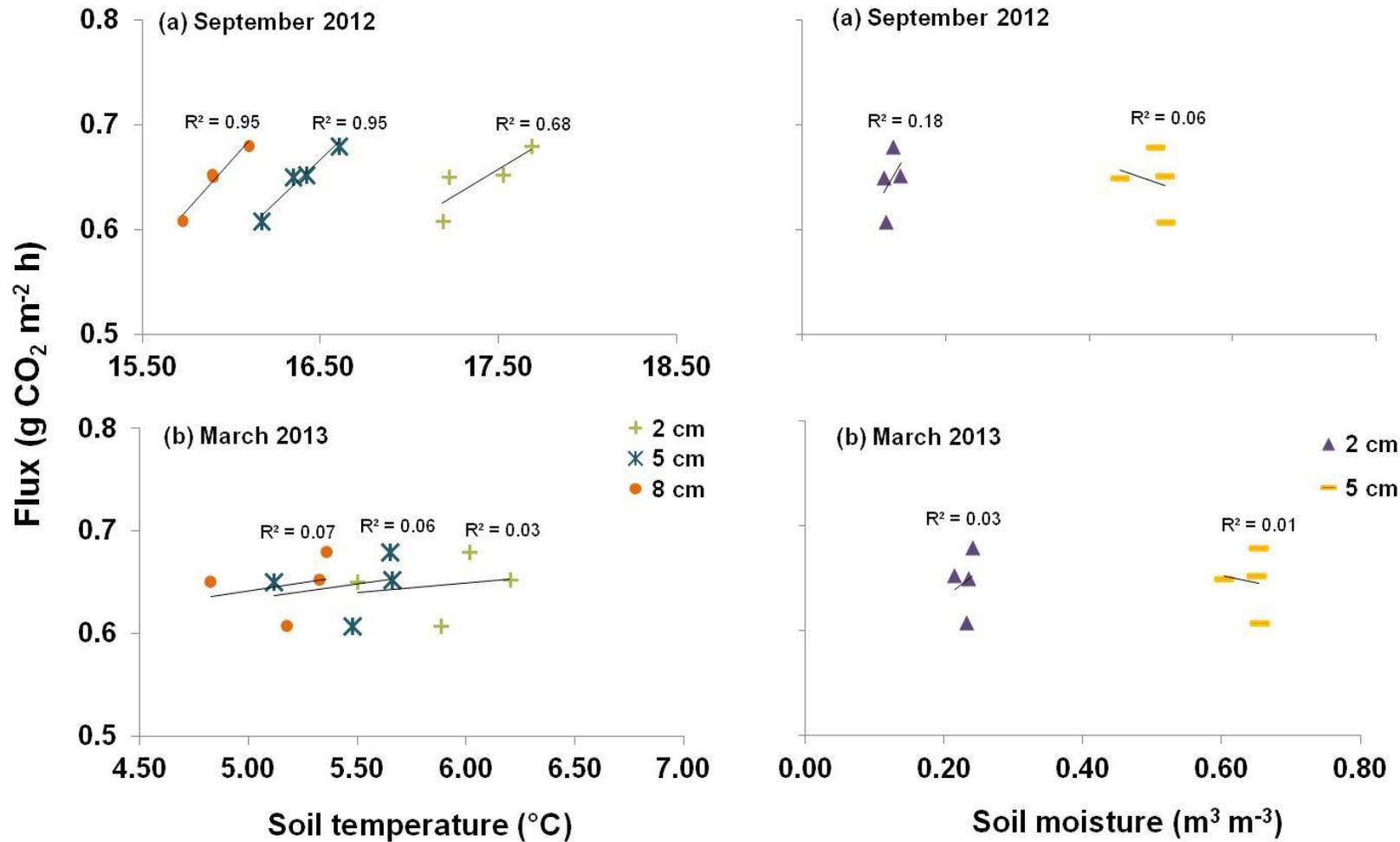


Figure B2-1. Correlations between soil flux and temperature and between soil flux and moisture, in September 2012 (a) and March 2013 (b)

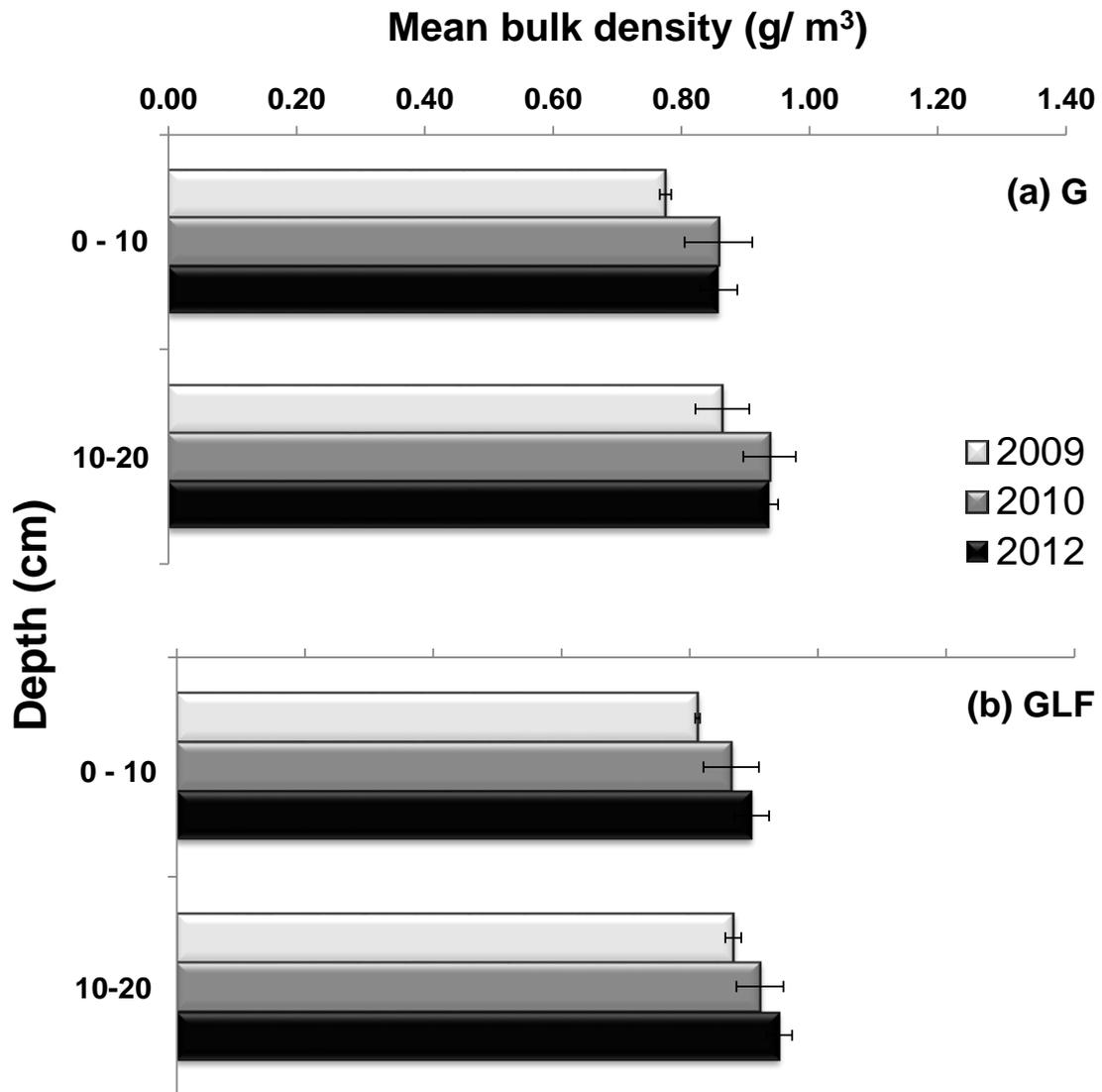


Figure B2-2. Soil mean bulk density with depth in the G and GLF plots, during three years of sampling taken by the team at NW

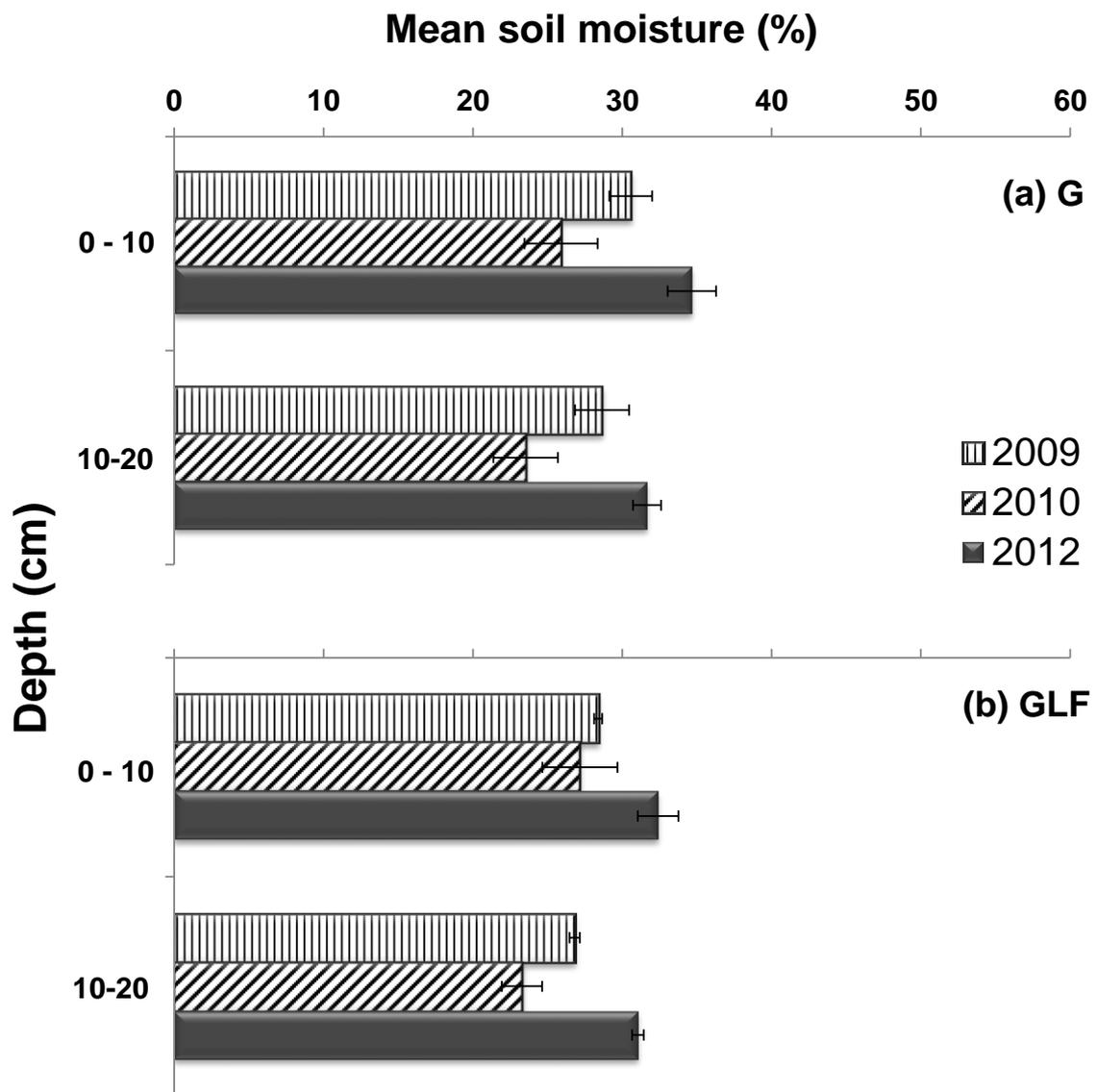


Figure B2-3. Mean soil moisture with depth in the G and GLF plots, during three years of sampling taken by the team at NW

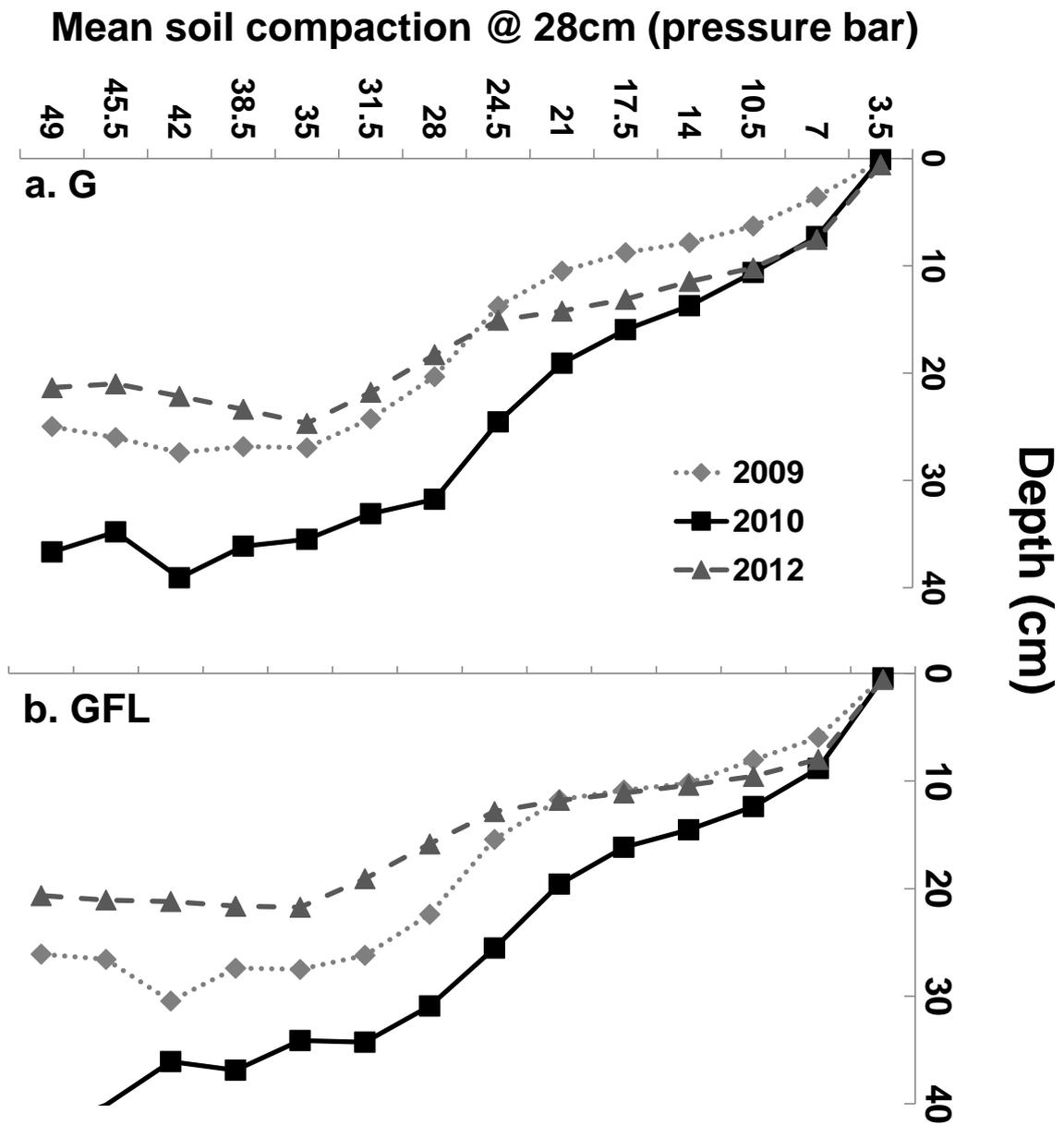


Figure B2-4. Mean soil compaction with depth in the G and GLF plots, during three years of sampling taken by the team at NW

B3. North Wyke Standard Laboratory Methods

Method B4-1. STM 246-05: Total Phosphorus in Water

 <p>NORTH WYKE LABORATORY</p> <p>NORTH WYKE RESEARCH</p>	Method	STM 246
	Issue No.	5
	Issue Date	21 st October 2014
<p>DETERMINATION OF TOTAL PHOSPHORUS IN WATER BY DISCRETE PHOTOMETRIC ANALYSIS</p>	Issued by	E R Dixon
	Pages	5

1 SCOPE

- 1.1 STM 246 describes the procedure for the determination of total phosphorus in raw and potable waters, including river water, rain water, waste water, surface and groundwater, using persulphate oxidation and a discrete photometric analyser. The limit of detection is $3.16\mu\text{gPO}_4\text{-P/litre}$ and the working range is $10 - 1500\mu\text{gPO}_4\text{-P/litre}$.
- 1.2 Non molybdate-reactive forms of phosphorus (organic, condensed and colloidal) are converted to orthophosphate by oxidation with acidified potassium persulphate in an autoclave at 121°C . Total phosphate is subsequently determined colorimetrically by reaction with ammonium molybdate in acid solution to form phosphomolybdic acid and reduction to phosphomolybdenum blue which has absorbance maxima at 660 and 880nm.
- 1.3 Silicon, at concentrations above about 4000mg/litre, would form silicomolybdic acid during colour development which would also give a blue colour when reduced. Tartrate is added to form a stable complex with molybdate which prevents the above reaction but allows the reaction with phosphate (Silicon concentrations are commonly between 0 and 20mg/litre in natural waters).
- 1.4 Concentrations of iron (III) greater than about 50mg/litre compete with the reducing agent ascorbic acid and thus cause a negative error. Pre-treatment of samples high in iron (III) with bisulphite is recommended. (Total iron concentrations in natural waters do not normally exceed 1mg/litre).

2 REFERENCE DOCUMENTS

- 2.1 EOP 219 Operation of Aquakem 250 analyser.
- 2.2 FO 62 STM 246 Worksheet.
- 2.3 Environment Agency National Laboratory Service Method of Analysis. No. SX1 Total Phosphorus, Issue 003, dated October 2008.
- 2.4 Murphy, J. and Riley, J.P. (1962). *A modified single solution method for the determination of phosphate in natural waters*. *Analytica Chimica Acta*, 27, 31-36.
- 2.5 Methods for the Examination of water and Associated Materials. Phosphorus in Waters, Sewage and Effluents 1981. ISBN 0 11 751582 5.

3 EQUIPMENT

- 3.1 Autoclave.
- 3.2 10ml borosilicate glass screw-neck culture tubes with PTFE-lined caps.
- 3.3 Aquakem 250 discrete photometric analyser.
- 3.4 Deionised water polisher to produce 18.2 megohm water.

4 REAGENTS (Reagent names in bold are those used in the Test Flow)

Use ultra-pure deionised water for the preparation of reagents and calibration standards.

- 4.1 Ammonium persulphate. Dissolve 50 ± 0.005 g ammonium persulphate $[(\text{NH}_4)_2\text{S}_2\text{O}_8]$, mol.wt. 228.20] in about 200mls ultra-pure deionised water in a 250ml graduated beaker and make up to the 250ml graduation mark with ultra-pure deionised water. Prepare on day of use and keep at room temperature to avoid precipitation.
- 4.2 Antimony potassium tartrate. Dissolve 0.340 ± 0.002 g antimony (III) potassium oxitartrate trihydrate $[\text{C}_4\text{H}_4\text{KO}_7\text{Sb}_3\text{H}_2\text{O}]$, mol.wt. 378.98], in about 50mls ultra-pure deionised water in a 100ml graduated beaker and make up to the 100ml graduation mark with ultra-pure deionised water. Stable in a refrigerator for one week.
- 4.3 Sulphuric acid (2.5M). In a fume cupboard, carefully, and with stirring, add 140mls concentrated sulphuric acid $[\text{H}_2\text{SO}_4]$, 98%, mol.wt. 98.07] to about 800mls ultra-pure deionised water in a one litre graduated beaker. Allow to cool before diluting to the 1000ml graduation mark with ultra-pure deionised water. Stable in a refrigerator for six months.
- 4.4 Aerosol solution (2%). Dilute 2mls of Aerosol 22 surfactant [tetrasodium {N-(1,2)} dicarboxyethyl)-N-octadecyl sulphosuccinamate] in about 80ml ultra-pure deionised water in a 100ml measuring cylinder or graduated beaker. Dilute to the 100ml graduation mark with ultra-pure deionised water and mix well. Stable in a refrigerator for three months.
- 4.5 Ammonium molybdate. Dissolve 3 ± 0.002 g ammonium molybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$, mol.wt. 1235.9] in about 100mls ultra-pure deionised water in a 250ml graduated beaker. Using a measuring cylinder add 75mls of 2.5M sulphuric acid and pipette in 25mls of antimony potassium tartrate solution. Make up to the 250ml graduation mark with ultra-pure deionised water. Stable in a refrigerator for one week.
- 4.6 TP – R1. To a 20ml Aquakem reagent tube add 20 ± 0.03 mls of the above ammonium molybdate solution and 0.5ml of 2% Aerosol solution, stopper and shake to mix.
- 4.7 TP – R2. Dissolve 3.5 ± 0.002 g L-ascorbic acid $[\text{C}_6\text{H}_8\text{O}_6]$, mol.wt. 176.13] in about 50mls ultra-pure deionised water in a 250ml graduated beaker and make up to the 200ml graduation mark with ultra-pure deionised water. Stable in a refrigerator for one week.

Appendices

Preparation of calibration standards:

Stock solution (Stable in a refrigerator for six months)	1000mgPO ₄ _P/litre	Dissolve 2.197 ± 0.002g potassium dihydrogen orthophosphate [KH ₂ PO ₄ , mol.wt. 136.09] in ultra-pure deionised water and make up to 500 ± 0.5mls with deionised water.
Working stock	10mgPO ₄ _P/litre	Dilute 10mls stock solution to 1000 ± 1mls with ultra-pure deionised water.
Calibration standards – stable in a refrigerator for three days.		
0 µg PO ₄ _P/litre	Ultra-pure deionised water.	
100 µg PO ₄ _P/litre	Dilute 1ml working stock to 100 ± 0.2mls with ultra-pure deionised water.	
200 µg PO ₄ _P/litre	Dilute 2mls working stock to 100 ± 0.2mls with ultra-pure deionised water.	
300 µg PO ₄ _P/litre	Dilute 3mls working stock to 100 ± 0.2mls with ultra-pure deionised water.	
400 µg PO ₄ _P/litre	Dilute 4mls working stock to 100 ± 0.2mls with ultra-pure deionised water.	
500 µg PO ₄ _P/litre	Dilute 5mls working stock to 100 ± 0.2mls with ultra-pure deionised water.	

5 PROCEDURES

Samples should be analysed as soon as practicable to minimise the risk of degradation, or stored at <10 °C in a refrigerator.

5.1 Digestion procedure

- 5.1.1 Into a 10ml digestion tube pipette 9 ± 0.02mls sample or calibration standard
- 5.1.2 Add 0.5ml of ammonium persulphate reagent and, from a dispenser, add 0.2ml concentrated sulphuric acid [H₂SO₄, 98%, mol.wt. 98.07].
- 5.1.3 Screw on teflon-lined cap, shake to mix and place in an autoclavable rack, noting positions on worksheet FO 62.
- 5.1.4 Autoclave at 195Kpa and 121oC for 30 – 35 minutes (See below). Allow to cool, leave caps on and transfer to Aquakem sample racks.
- 5.1.5 Place racks in an ultrasonic bath for at least 5 minutes.
- 5.1.6 Place piece of paper between the two rows of tubes to fool the barcode reader.

5.2 Autoclave

Currently using Astell autoclave in laboratory 117.

- 5.2.1 If requested, pour about 500mls tap water into the back.
- 5.2.2 Place sample racks inside and shut door.
- 5.2.3 Select programme 3 (121oC for 30 minutes) and Start.

5.2.4 After about 1½ hours press Open (note 30 second delay before door unlocks) and remove samples.

5.3 Aquakem analyser

For setting up the analyser, calibration, running samples and shutting down, refer to equipment operating procedure EOP 219.

Operational Note: If a digest is diluted by the analyser, then it should be diluted manually and re-analysed.

APPENDIX 1 - METHOD VALIDATION

Data source	Assessment	µgPO ₄ _P/litre
Validation folder	Limit of detection (LOD)	3.16
Validation folder	Limit of quantification (LOQ)	10.07
Validation folder	Working range	10 – 1500
AQC/534, 535 & 536	Accuracy/Bias	99%
Validation folder	Within-run precision (RSD%)	1.4%
AQC/534, 535 & 536	Within-lab precision (RSD%)	5%
Uncertainty file	Uncertainty - 95% Confidence limit	± 19%
Validation folder	Recovery from spiked rain water	84.3%
Validation folder	Recovery from spiked river water	89.7%
Validation folder	Recovery from spiked field drainage	80.7%

APPENDIX 2 - BLANK VALUES AND PURITY OF DEIONISED WATER

Average values for blanks prepared from either Elga reverse osmosis water or Milli-Q ultra pure 18.2 megohm water were:

- Elga reverse osmosis water - 29.442 µg PO₄_P / litre (N = 28), rel. std. deviation 3.2%
- Milli-Q ultra pure water - 7.093 µg PO₄_P / litre (N = 28), rel. std. deviation 8.3%

Appendices

APPENDIX 2 – TEST PARAMETERS

Name of test	Total P in waters 0-0.5mg/l				
Online name	TP 0-500	Test in Use	YES		
Test type	Photometric		LOW	HIGH	
Results unit	µg/l	Test limit	*	1500 µg/l	
Number of decimals	3	Initial absorbance	*	A	
		Dilution limit	*	500 µg/l	
		Secondary dilution	0.0	2.0	
		Critical limit	*	*µg/l	
		Reflex test limit	*	*µg/l	
		Reflex test			
Acceptance	Manual	Reference class	LOW	HIGH	In use
Dilution 1+	0.0				
Sample type	Water	Correction factor	1.00		
	Raw water	Correction bias	0.0	µg/l	
	Sewage				
Calibration type		Polynomial			
Curve direction		Ascending			
Repeat time (d)		30	Abs error (mA)	*	
Points/cal.		Single	rel error (%)	*	
Acceptance		Manual			
Response limit (mA)		MIN	MAX		
		*	*		
Coefficient of determination		0.9994			
Bias correction in use		NO			
Cd reduction		NO			
Type of Calibrators		Separate			
Calibrator		Conc.	Dil. Ratio		
TP - 0		0.000	1+0.0		
TP - 100		100.000	1+0.0		
TP - 200		200.000	1+0.0		
TP - 300		300.000	1+0.0		
TP - 400		400.000	1+0.0		
TP - 500		500.000	1+0.0		
Manual QC in Use		NO	Routine QC in use	NO	
Blank		YES	Normal cuvette		
Sample			Volume (µl)	120	
Disp. With		Extra	Add Volume (µl)	40	
Dilution with		Water	Wash reagent	None	
Measurement		End point	Blank		
Resp. Min (A)		*	Resp. Max (A)	*	
Reagent		TP – R1	Volume (µl)	35	
Disp. with		Extra	Add. Volume (µl)	30	
Wash reagent		None			
Syringe speed		Normal			
Incubation			Time (sec)	120	
Reagent		TP – R2	Volume (µl)	15	
Disp. with		Extra	Add. Volume (µl)	20	
Wash reagent		None			
Syringe speed		Normal			
Incubation			Time (sec)	540	
Measurement		End point			
Wavelength (nm)		880nm	Side wavelength (nm)	None	
Meas. type		Normal			

Method B4-2. STM 247-01: Total Organic Nitrogen (TON) in Water

 NORTH WYKE LABORATORY NORTH WYKE RESEARCH	Method	STM 247
	Issue No.	1
DETERMINATION OF TOTAL OXIDISED NITROGEN IN WATER BY DISCRETE PHOTOMETRIC ANALYSIS	Issue Date	11th May 2010
	Issued by	A W Bristow
	Pages	5

1 SCOPE

- 1.1 STM 247 describes the procedure for the determination of total oxidised nitrogen in raw and potable waters, including river water, rain water, waste water, surface and groundwater, using a discrete photometric analyser. The limit of detection is 0.06 and the working range is 0.07 – 250 mgTON/litre.
- 1.2 Nitrate is reduced to nitrite by hydrazine sulphate and total nitrite is diazotized with sulphanilamide and coupled with N-1-naphthylethylenediamine dihydrochloride to form an azo dye with an absorbance maximum at 540nm.

2 REFERENCE DOCUMENTS

- 2.1 EOP 219 Operation of Aquakem 250 analyser.
- 2.2 Aquakem method TON, issue 2, dated 1st January 2006.
- 2.3 Kamphake, L.J., Hannah, S.A. and Cohen, J.M. (1967). Automated analysis for nitrate by hydrazine reduction. Water Research Laboratory, **1967**, 205-216.
- 2.4 Kempers, A.J. and Luft, A.G. (1988). Re-examination of the determination of environmental nitrate as nitrite by reduction with hydrazine. Analyst, **113**, 1117-1120.

3 EQUIPMENT

- 3.1 Aquakem 250 discrete photometric analyser.

4 REAGENTS (Reagent names in bold are those used in the Test Flow)

- 4.1 Copper II sulphate solution. Dissolve 0.780 ± 0.002 g copper sulphate [CuSO₄.5H₂O, mol.wt. 249.68] in ultra-pure deionised water in a 200ml volumetric flask and make up to the mark with ultra-pure deionised water. Transfer to a dark bottle. Stable in a refrigerator for one month.
- 4.2 Zinc sulphate solution. Dissolve 9.000 ± 0.002 g zinc sulphate [ZnSO₄.7H₂O, mol.wt. 287.54] in ultra-pure deionised water in a 200ml volumetric flask and make up to the mark with ultra-pure deionised water. Transfer to a dark bottle. Stable in a refrigerator for one month.
- 4.3 TONR1 (Aquakem Reagent TON 1). Dissolve 0.8g sodium hydroxide [NaOH, mol.wt. 40.00] in ultra-pure deionised water in a 100ml volumetric flask and make up to the mark with ultra-pure deionised water. Stable in a refrigerator for one week.
- 4.4 TONR2 (Aquakem Reagent TON 2). Dissolve 0.325 ± 0.002 g hydrazine sulphate [N₂H₆SO₄, mol.wt. 130.12] in about 400mls ultra-pure deionised water in a 500ml graduated beaker. Add 0.75ml copper sulphate solution and 5mls

Appendices

zinc sulphate solution and make up to the 500ml mark with ultra-pure deionised water. Stable in a refrigerator for one month.

- 4.5 TONR3 (Aquakem reagent TON 3). Slowly, and with stirring, pipette 50 ± 0.050 mls orthophosphoric acid [H_3PO_4 , mol.wt. 98.00, 85%, sp.gr. 1.7] to about 500mls ultra-pure deionised water in a 1000ml graduated beaker. Add 5 ± 0.002 g sulphanilamide [$\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$, mol.wt. 172.21] and dissolve completely before adding 0.25 ± 0.002 g N-1-naphthylethylenediamine dihydrochloride [$\text{C}_{10}\text{H}_7\text{NH.CH}_2\text{CH}_2\text{NH}_2\cdot 2\text{HCl}$, mol.wt. 259.18]. Dilute to the 1000ml mark with ultra-pure deionised water and transfer to a dark bottle. Stable in a refrigerator for one month.
- 4.6 Preparation of calibration standards.
 - 4.6.1 Calibration stock (1000mgTON/litre). Dissolve $3.034\text{g} \pm 0.002\text{g}$ sodium nitrate [NaNO_3 , mol.wt. 84.99] in ultra-pure deionised water in a 500ml volumetric flask and make up to the mark with ultra-pure deionised water. Stable in a refrigerator for one month.
 - 4.6.2 $\text{NO}_3\text{-}20$ (20mgTON/litre). Dilute 2mls calibration stock to 100 ± 0.2 mls with ultra-pure deionised water in a 100ml volumetric flask.
 - 4.6.3 $\text{NO}_3\text{-}100$ (100mgTON/litre). Dilute 10mls calibration stock to 100 ± 0.2 mls with ultra-pure deionised water in a 100ml volumetric flask.

5 PROCEDURES

- 5.1 Samples should be analysed as soon as practicable to minimise the risk of degradation, or stored at $<10^\circ\text{C}$ in a refrigerator.
- 5.2 For setting up the analyser, calibration, running samples and shutting down, refer to equipment operating procedure EOP 219.
- 5.3 Note that the method can reflex from a low concentration range to a high concentration range so both calibrants may be required.

APPENDIX 1 - METHOD VALIDATION

Data source	Assessment	mgTON/litre
Validation folder	Limit of detection (LOD)	0.06
Validation folder	Limit of quantification (LOQ)	0.07
Validation folder	Working range	0.07 – 250
AQC and Aquacheck data	Accuracy/Bias	100.4%
Validation folder	Within-run precision (RSD%)	2.6%
AQC/421 & 422	Within-lab precision (RSD%)	5.3%
Uncertainty file	Uncertainty - 95% Confidence limit	$\pm 11.8\%$
Validation folder	Recovery from spiked rain-water	105.2%
Validation folder	Recovery from spiked river-water	101.6%
Validation folder	Recovery from spiked field-drainage	108.4%
Validation folder	Recovery from spiked sea-water	104.1%

APPENDIX 2 – TEST PARAMETERS FOR 0-1mgTON/LITRE IN WATER

Name of test	Total oxidised nitrogen				
Online name	TON 0-1	Test in Use	YES		
Test type	Photometric		LOW	HIGH	
Results unit	mg/l	Test limit	0	3mg/l	
Number of decimals	3	Initial absorbance	*	* A	
		Dilution limit	*	1 mg/l	
		Secondary dilution 1+	0.0	2.0	
		Critical limit	*	* mg/l	
		Reflex test limit	*	* mg/l	
		Reflex test			
Acceptance Dilution 1+	Manual 0.0	Reference class	LOW	HIGH	In use
Sample type	Water	Correction factor	1.00		
	Raw water	Correction bias	0.0	mg/l	
	Sewage				
Calibration type	Polynomial				
Curve direction	Ascending				
Repeat time (d)	0	Abs error (mA)	*		
Points/cal.	Single	rel error (%)	*		
Acceptance	Manual				
Response limit (mA)	MIN	MAX	*		
	*	*			
Coeff. Of determination	0.99900				
Bias correction in use	NO				
Cd reduction	NO				
Type of Calibrators	Series				
Calibrator	Conc	Dil. Ratio			
S-NO3-0	0.000	1+0.0			
S-NO3-5	5.000	1+ 19.0			
S-NO3-5	5.000	1+17.0			
S-NO3-5	5.000	1+14.0			
S-NO3-5	5.000	1+11.0			
S-NO3-5	5.000	1+9.0			
S-NO3-5	5.000	1+7.0			
S-NO3-5	5.000	1+4.0			
Manual QC in Use	NO	Routine QC in use	NO		
Blank	YES				
Reagent	TONR1	Normal cuvette			
Disp. with	Extra	Volume (µl)	50		
Wash reagent	None	Add. Volume (µl)	40		
Syringe speed	Normal				
Sample		Volume (µl)	10		
Disp. With	Water	Add Volume (µl)	40		
Dilution with	Water	Wash reagent	None		
Incubation		Time (sec)	180		
Measurement	End point	Blank			
Resp. Min (A)	*	Resp. Max (A)	*		
Reagent	TONR2	Volume (µl)	65		
Disp. with	Extra	Add. Volume (µl)	40		
Wash reagent	None				
Syringe speed	Normal				
Incubation		Time (sec)	420		
Reagent	TONR3	Volume (µl)	30		
Disp. with	Extra	Add. Volume (µl)	40		
Wash reagent	None				
Syringe speed	Normal				
Incubation		Time (sec)	300		
Measurement	End point				
Wavelength (nm)	540nm	Side wavelength (nm)	None		
Meas. type	Normal				

Appendices

APPENDIX 3 – TEST PARAMETERS FOR 0-5mgTON/LITRE IN WATER

Name of test	Total oxidised nitrogen	Test in Use	YES	
Online name	TON 0-5		LOW	HIGH
Test type	Photometric	Test limit	0	*
Results unit	mg/l	Initial absorbance	*	A
Number of decimals	3	Dilution limit	*	5 mg/l
		Secondary dilution 1+	0.0	0.0
		Critical limit	*	* mg/l
		Reflex test limit *		5 mg/l
		Reflex test	TON 5-50	
Acceptance	Manual	Reference class	LOW	HIGH
Dilution 1+	0.0			In use
Sample type	Water	Correction factor	1.00	
	Raw water	Correction bias	0.0	mg/l
	Sewage			
Calibration type	Polynomial			
Curve direction	Ascending			
Repeat time (d)	0	Abs error (mA)	*	
Points/cal.	Single	rel error (%)		
Acceptance	Manual			
Response limit (mA)	MIN	MAX		
	*	*		
Coeff. Of determination	0.99900			
Bias correction in use	NO			
Cd reduction	NO			
Type of Calibrators	Series			
Calibrator	Conc.	Dil. Ratio		
S-NO3-0	0.000	1+0.0		
S-NO3-20	20.000	1+ 39.0		
S-NO3-20	20.000	1+19.0		
S-NO3-20	20.000	1+11.5		
S-NO3-20	20.000	1+9.0		
S-NO3-20	20.000	1+4.0		
S-NO3-20	20.000	1+4.0		
S-NO3-20	20.000	1+3.0		
Manual QC in Use	NO	Routine QC in use	NO	
Blank	YES	Normal cuvette		
Reagent	TONR1	Volume (µl)	50	
Disp. with	Extra	Add. Volume (µl)	40	
Wash reagent	None			
Syringe speed	Normal			
Sample		Volume (µl)	10	
Disp. With	Water	Add. Volume (µl)	40	
Dilution with	Water	Wash reagent	None	
Incubation		Time (sec)	180	
Measurement	End pointBlank			
Resp. Min (A)	*	Resp. Max (A)	*	
Reagent	TONR2	Volume (µl)	65	
Disp. with	Extra	Add. Volume (µl)	40	
Wash reagent	None			
Syringe speed	Normal			
Incubation		Time (sec)	420	
Reagent	TONR3	Volume (µl)	30	
Disp. with	Extra	Add. Volume (µl)	40	
Wash reagent	None			
Syringe speed	Normal			
Incubation		Time (sec)	300	
Measurement	End point			
Wavelength (nm)	540nm	Side wavel. (nm)	None	
Meas. type	Normal			

APPENDIX 4 – TEST PARAMETERS FOR 5-50mgTON/LITRE IN WATER

Name of test	Total oxidised nitrogen	Test in Use	YES	
Online name	TON 5-50		LOW	HIGH
Test type	Photometric	Test limit	0	150 mg/l
Results unit	mg/l	Initial absorbance	*	A
Number of decimals	3	Dilution limit	*	50 mg/l
		Secondary dilution 1+	0.0	2.0
		Critical limit	*	* mg/l
		Reflex test limit	*	* mg/l
		Reflex test	*	*
Acceptance	Manual	Reference class	LOW	HIGH In use
Dilution 1+	0.0			
Sample type	Water	Correction factor	1.00	
	Raw water	Correction bias	0.0	mg/l
	Sewage			
Calibration type	Polynomial			
Curve direction	Ascending			
Repeat time (d)	0	Abs error (mA)	*	
Points/cal.	Single	rel error (%)		
Acceptance	Manual			
Response limit (mA)	MIN	MAX		
	*	*		
Coeff. Of determination	0.99900			
Bias correction in use	NO			
Cd reduction	NO			
Type of Calibrators	Series			
Calibrator	Conc.	Dil. Ratio		
S-NO3-100	100.000	1+19.0		
S-NO3-100	100.000	1+ 9.0		
S-NO3-100	100.000	1+4.0		
S-NO3-100	100.000	1+2.0		
S-NO3-100	100.000	1+1.5		
S-NO3-100	100.000	1+1.0		
Manual QC in Use	NO	Routine QC in use	NO	
Blank	YES			
Reagent	TONR1	Normal cuvette		
Disp. with	Extra	Volume (µl)	50	
Wash reagent	None	Add. Volume (µl)	40	
Syringe speed	Normal			
Sample		Volume (µl)	4	
Disp. With	Water	Add. Volume (µl)	46	
Dilution with	Water	Wash reagent	None	
Incubation		Time (sec)	180	
Measurement	End pointBlank			
Resp. Min (A)	*	Resp. Max (A)	*	
Reagent	TONR2	Volume (µl)	65	
Disp. with	Extra	Add. Volume (µl)	40	
Wash reagent	None			
Syringe speed	Normal			
Incubation		Time (sec)	420	
Reagent	TONR3	Volume (µl)	30	
Disp. with	Extra	Add. Volume (µl)	40	
Wash reagent	None			
Syringe speed	Normal			
Incubation		Time (sec)	300	
Measurement	End point			
Wavelength (nm)	540nm	Side wavel. (nm)	None	
Meas. type	Normal			

Appendices

Method B4-3. STM 241-03: Total Phosphorus in Soil

 <p>NORTH WYKE LABORATORY</p> <p>NORTH WYKE RESEARCH</p>	Method	STM 214
	Issue No.	3
	Issue Date	21 st October 2014
<p>DETERMINATION OF TOTAL PHOSPHATE IN SOILS BY SPECTROPHOTOMETRY</p>	Issued by	E R Dixon
	Page	228 of 46

1 SCOPE

- 1.1 STM 214 describes the procedure for the determination of total phosphate in dry soil by fusion with sodium hydroxide. The limit of detection is 1.2µg PO₄_P (160mgP/kg dry soil) and the working range 550 – 20000mgP/kg dry soil.
- 1.2 Soil is fused with sodium hydroxide and the melt taken up in water. After centrifugation and adjustment of the pH to 6, phosphate is determined colorimetrically as molybdenum blue. There are no significant interferences.

2 REFERENCE DOCUMENTS

- 2.1 EOP 207 Operation of Unicam UV2 spectrophotometer
- 2.2 EOP 222 Operation of Cecil Instrument CE 2021 spectrophotometer
- 2.3 FO42 STM 214 Worksheet – Determination of total phosphate in soil
- 2.4 FO46 Spectrophotometer Performance Checks
- 2.5 FO48 Drying to Constant Weight

3 EQUIPMENT

- 3.1 Bunsen burner
- 3.2 Tripod
- 3.3 Triangle
- 3.4 50 ml nickel crucibles
- 3.5 Spectrophotometer set at 880nm
- 3.6 Centrifuge, 3000 rev/minute
- 3.7 Deionised water polisher to produce 18.2 megohm water.

4 REAGENTS (use ultra-pure deionised water)

4.1 Preparation of reagents

- 4.1.1 Nitrophenol indicator. Dissolve 0.2 ± 0.02 g GPR 3-nitrophenol [NO₂.C₆H₄.OH, mol. wt. 139.11] in 100 ml water. Stable for up to 3 months in a dark bottle in a refrigerator.
- 4.1.2 2.5M sulphuric acid. Add 70 ± 1 ml AR concentrated sulphuric acid [H₂SO₄, mol. wt. 98.07, sp.gr. 1.84] slowly and with stirring to about 300 ml water in a 500 ml graduated beaker. Make up to 500 ± 5 ml and transfer to a reagent bottle. Stable in a refrigerator for up to 6 months.
- 4.1.3 Ammonium molybdate solution. Dissolve 20 ± 0.05 g AR ammonium molybdate [(NH₄)₆Mo₇O₂₄.4H₂O, mol. wt. 1235.9], in water in a volumetric flask and dilute to 500 ± 0.25 ml. Stable in a refrigerator for up to 3 months.
- 4.1.4 Ascorbic acid (0.1M). Weigh 1.321 ± 0.002 g AR ascorbic acid [C₆H₈O₆, mol. wt. 176.13] into a 100 ml reagent bottle or volumetric flask, add 75 ± 1 ml water and shake to dissolve. Prepare on day of analysis. Do not store.
- 4.1.5 Antimony potassium tartrate (1 mg Sb/ml). Dissolve 0.311 ± 0.002 g antimony (III) potassium oxitartrate trihydrate [C₄H₄KO₇Sb.3H₂O, mol.wt. 378.98] in water in a volumetric flask and dilute to 100 ml. Ensure that the antimony potassium tartrate is completely dissolved. Stable in a refrigerator for up to one month.
- 4.1.6 Mixed reagent. Using measuring cylinders, mix 125 ml 2.5M sulphuric acid with 37.5 ml ammonium molybdate solution in a beaker. Add the entire quantity (75 ml) ascorbic acid solution and 12.5 ml antimony potassium tartrate solution. Prepare fresh each day.

4.2 Preparation of calibration standards (use ultra-pure deionised water)

- 4.2.1 Stock phosphate solution (50 µg P/ml). Dissolve 0.220 ± 0.001 g AR potassium dihydrogen orthophosphate, [KH₂PO₄, mol. wt. 136.09] in water in a volumetric flask and make up to 1 litre. Store in a refrigerator for up to 6 months.
- 4.2.2 Working stock solution (0.5 µg P/ml). Dilute 10 ± 0.02 ml stock phosphate solution to 1 litre with water. Discard after use.
- 4.2.3 Prepare calibration standards (use ultra-pure deionised water)

Calibration standards (Prepare on day of analysis. Not stable, discard after use)	
0 µg in 50 ml volumetric flask	Approximately 30ml water in a 50 ml volumetric flask
5 µg in 50 ml volumetric flask	Add 10ml working stock a 50 ml volumetric flask
10 µg in 50 ml volumetric flask	Add 20ml working stock a 50 ml volumetric flask
15 µg in 50 ml volumetric flask	Add 30ml working stock a 50 ml volumetric flask
20 µg in 50 ml volumetric flask	Add 40ml working stock a 50 ml volumetric flask

- 4.2.4 For colour development see section 5.3.

Appendices

5 PROCEDURES

Include analytical quality control (AQC) standards AQC/371, AQC/372 and where possible, run duplicates of samples. AQC's and duplicates together must comprise at least 10 percent of a run. Use worksheet FO42 to record the results.

5.1 Sample preparation

Dry soil at 25 ± 10 °C to constant weight, using form FO48 to record the details, and ball-mill for 5 minutes to a fine powder.

5.2 Fusion

5.2.1 Weigh accurately $0.1-0.25 \pm 0.001$ g soil into a 50 ml nickel crucible and add $1\frac{3}{4}-2\frac{1}{2}$ g NaOH. Heat gently for about 5 minutes over a bunsen, swirling the crucible to mix, and avoiding losses through spitting. Take care with soils containing more than 10% organic matter. Include reagent blanks containing sodium hydroxide only.

5.2.2 Immediately place on a pipeclay triangle supported on a tripod and heat to a dull red heat using a hotter bunsen, for a further 5 minutes to ensure complete decomposition. Allow to cool on an asbestos mat for at least 2 minutes.

5.2.3 When cold, add about 40 ml ultra-pure deionised water to the crucible and allow to stand for 2 hours.

5.2.4 Quantitatively transfer the contents of the crucible to a 100 ml volumetric flask, breaking up the melt with a glass rod, and make up to the mark with ultra-pure deionised water.

5.2.5 Shake the flask and transfer about 12mls to a plastic centrifuge tube. Centrifuge at a displayed value of 3,000 revs/min for about 10 minutes. Transfer the supernatant to a sample vial. Samples can be stored in a refrigerator at this stage.

5.3 Colour development

5.3.1 Transfer 1-10 ml (normally 5 ml) of the supernatant to a 50 ml volumetric flask.

5.3.2 Dilute to about 30mls with ultra-pure deionised water.

5.3.3 Add 2-3 drops of 3-nitrophenol indicator solution and adjust to pH 6 by the drop-wise addition of 2.5M sulphuric acid (yellow to colourless end-point).

5.3.4 Add 4 ± 0.02 ml mixed reagent, mix and make up to the mark with ultra-pure deionised water. Allow 30 minutes for colour development (colour stable for 24 hours).

5.4 Calibration

Set up the spectrophotometer according to appropriate Equipment Operating Procedure, zero the instrument on water and run a linear calibration using the calibration standards described in section 4.2.3.

5.5 Running samples

Measure absorption at 880 nm using water as reference, and record μgP present on FO42.

5.6 Calculation of results

$$\text{Total soil phosphorus } (\mu\text{g P/g}) = \frac{(\mu\text{g P present} - \text{mean blank}) \times 100}{\text{weight soil taken(g)} \times \text{volume digest taken (ml)}}$$

The blank should not exceed 0.5 $\mu\text{gPO}_4\text{-P}$ present. A high blank is indicative of contamination.

5.6.1 Open the template STM 214_template.xlt in \\nwnetapp1a\selborne\TEMPLATES.

5.6.2 Transfer the relevant data from the worksheet into the appropriate columns.

5.6.3 Save the template file as boxnumber_your initials.xls in \\nwnetapp1a\selborne\DATA FILES\Total P in Soil.

6 REFERENCES

Smith, B.F.L. and Bain, D.C. (1982). A sodium hydroxide fusion method for the determination of total phosphate in soils. *Communications in Soil Science and Plant Analysis*, **13** (3), 185-190.

Murphy, J. and Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31-36.

Appendices

APPENDIX 1 - METHOD VALIDATION

Data source	Assessment	µg PO₄_P
Validation file	Limit of detection (LOD)	1.2 µg PO ₄ _P (160mgP/kg dry soil)
Validation file	Limit of quantification (LOQ)	6.1 µg PO ₄ _P (550mgP/kg dry soil)
Validation file	Working range	550-20000mgP/kg dry soil
Validation file	Correlation coefficient (R ²)	0.99872
Certified ref. soils	Accuracy/Bias	+ 9.5 %
Validation file	Within-run precision (RSD)	0.16
Validation file	Within-lab precision (RSD)	0.15
Uncertainty file	Uncertainty - 95% Confidence limit	± 41%

APPENDIX 2 - METHOD COMPARISON

Comparison of results from sodium hydroxide fusion method (North Wyke) and Aqua Regia digestion (Rothamsted) for soils from NW532, North Wyke Laboratory registration 12817.

Sample ID	mg Total P / kg dry soil		% difference
	North Wyke	Rothamsted	
NW532/2	491	336	-31%
NW532/3	506	309	-39%
NW532/1	601	388	
NW532/7	660	522	
NW532/29	713	554	
NW532/11	771	694	
NW532/14	842	869	
NW532/15	894	872	
NW532/39	1146	1032	
NW532/34	1199	1117	
NW532/37	1211	1086	
NW532/40	1295	1123	

Method B4-4. STM 219-03: Soil and Water pH

 <p>NORTH WYKE LABORATORY</p> <p>NORTH WYKE RESEARCH</p>	Method	STM 219
	Issue No.	3
<p>DETERMINATION OF pH IN SOIL AND WATER</p>	Issue Date	17 May 11
	Issued by	A W Bristow
	Pages	46

1 SCOPE

- 1.1 STM 219 describes the procedure for the determination of pH in environmental water samples, dry soils and slurry using a pH meter
- 1.2 Measurement of pH is made by comparing the emf output of an electrochemical cell comprising glass electrode/test solution/reference electrode with the emf output of the same cell system containing a standard solution of known pH in place of the test solution.

If the test and reference solutions are at the same temperature, the pH of the test solution can be calculated from the emf data according to the equation:

$$pH_{\text{sample}} = pH_{\text{standard}} + \frac{(E_{\text{sample}} - E_{\text{standard}})}{kT}$$

where k is a constant
 T is the absolute temperature °K
 E is the measured emf

- 1.3 In practice, pH meters provide electronic conversion of the kT factor, enabling a direct readout of pH.

2 REFERENCE DOCUMENTS

- Jenway Model 3320 pH meter Operating Manual (Reference 546 006/REV A/03/96)
- EOP 217 Operation of Jenway model 3320 pH meter.
- Instruction manual – HI9811-0 • HI9811-05 portable pH/°C /EC/TDS meters.
- EOP 208 Operation of HANNA HI9811-05 pH/°C /EC/TDS meter.
- FO07 Performance checks – pH meter
- FO25 STM 219 Worksheet – Determination of pH in soil and water.

3 EQUIPMENT

- 3.1 pH meter fitted with general purpose combination electrode.
- 3.2 Scoop with nominal capacity 10 ml.
- 3.3 Timer.

Appendices

4 REAGENTS

- 4.1 'Colourkey' buffer solution (red), pH 4.0 ± 0.02 at 20°C . (VWR product 19239 5W)
- 4.2 'Colourkey' buffer solution (yellow), pH 7.0 ± 0.02 at 20°C . (VWR product 19240 5H)
- 4.3 'Colourkey' buffer solution (blue), pH 10.0 ± 0.05 at 20°C . (VWR product 19241 5J)

5 PROCEDURES

Include analytical quality control (AQC) standards and, where possible, run duplicates of some samples. AQC's and duplicates together must comprise at least ten percent of a run.

5.1 Sample preparation

Water samples should be stored at $<10^{\circ}\text{C}$ and brought to room temperature before analysis. Soils should be dried to constant weight at $25 \pm 5^{\circ}\text{C}$ before being ground to pass a 2mm sieve.

5.2 Setting up the pH meter

Set up the pH meter according to EOP 208 or 217 and record performance checks on FO07.

5.3 pH in water

- 5.3.1 Place the pH electrode in the gently stirred sample and record the pH when the stability symbol appears.
- 5.3.2 Record the values on FO25.

5.4 Soil pH, ADAS method (ADAS, 1986)

- 5.4.1 Over-fill a 10 ml scoop with air-dried soil, ground to pass a 2mm sieve, strike off level without tapping, and transfer to a sealable plastic bag or plastic bottle.
- 5.4.2 Add 25 ± 0.1 ml freshly drawn deionised water, seal the bag or cap the bottle and shake for 15 ± 1 minutes.
- 5.4.3 Insert the electrode gently into the soil/water paste, swirl briefly and record the pH when the stability symbol appears.
- 5.4.4 Record the values on FO25.

- 5.5 Refer to EOP 208 and EOP 217 to shut down the pH meter.

6 REFERENCES

The Analysis of Agricultural Materials – *Method 32: pH and Lime Requirement of Mineral Soil*; Ministry of Agriculture, Fisheries and Food reference book 427 (1986).

Hess, P.R. (1971), *A Textbook of Soil Chemical Analysis*.

Scofield and Taylor (1955), Soil Science Society of America Proceedings, **19**, 164 – 167. Methods of Soil Analysis, part 2 – Chemical and Microbiological Properties. p923.

APPENDIX 1- METHOD VALIDATION**Water Analysis**

Data source	Assessment	pH
	Limit of detection (LOD)	Not applicable
	Limit of quantification (LOQ)	Not applicable
Calibration standards	Working range	4 - 10
	Correlation coefficient	Not applicable
Aquacheck dists 153-317	Accuracy/bias	-0.6%
LFN 00523, p 76	Within-run precision (RSD)	0.004
AQC/381 - 382	Within-lab precision (RSD)	0.008
Aquacheck dists 153-317	Between-lab precision (RSD)	0.009
Uncertainty file	Uncertainty – 95% Confidence limit	± 2%

Soil Analysis

Data source	Assessment	pH
	Limit of detection (LOD)	Not applicable
	Limit of quantification (LOQ)	Not applicable
Calibration standards	Working range	4 - 10
	Correlation coefficient	Not applicable
	Accuracy/bias	No data
LFN 00523, p 99-100	Within-run precision (RSD)	0.022
AQC/37 & 208	Within-lab precision (RSD)	0.015
	Between-lab precision (RSD)	No data
Uncertainty file	Uncertainty – 95% Confidence limit	± 6.5%

Appendices

Method B4-5. STM 250-03: Olsen Phosphorus

 <p>NORTH WYKE LABORATORY NORTH WYKE RESEARCH</p>	Method	STM 250
	Issue No.	3
	Issue Date	21 st October 2014
<p>DETERMINATION OF OLSEN P IN SOIL BY EXTRACTION AND DISCRETE PHOTOMETRIC ANALYSIS</p>	Issued by	E R Dixon
	Pages	46

1 SCOPE

- 1.1 STM 250 describes the procedure for the determination of Olsens's plant-available phosphorus in soil by extraction with sodium hydrogen carbonate solution followed by analysis using a discrete photometric analyser.
- 1.2 Soil is extracted with 0.5M sodium hydrogen carbonate solution adjusted to pH 8.5. After acidification of the filtered extract to remove carbon dioxide, the sample is reacted with molybdate and ascorbic acid, catalysed by antimony potassium tartrate, to form phosphomolybdenum blue, with absorbance maxima at 660 nm and 880 nm.
- 1.3 There is no universal extraction procedure which accurately estimates plant-available phosphorous (predominantly phosphates of calcium, iron and aluminium) for all crops and on all types of soil. This method correlates quite well with plant uptake in many soils.

2 REFERENCE DOCUMENTS

- 2.1 EOP 219 Operation of Aquakem 250 analyser.
- 2.2 M.A.F.F. Reference Book 427. The Analysis of Agricultural Materials. Method 59, Phosphorus, Extractable, in Soil. pp 183-185.
- 2.3 Murphy, J. and Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31-36.
- 2.4 Olsen S.R., Cole C.V., Watanabe F.S. and Dean L.A. (1954). Estimation of Available Phosphorus in Soils by Extraction With Sodium Bicarbonate. United States Department of Agriculture Circular no.959.

3 EQUIPMENT

- 3.1 Aquakem 250 discrete photometric analyser.
- 3.2 Reciprocal shaker.
- 3.3 Deionised water polisher to produce 18.2 megohm water.

4 REAGENTS (Reagent names in bold are those used in the Test Flow)

- 4.1 Sulphuric acid (~1.5M). Add 16 ± 0.5mls concentrated sulphuric acid [H₂SO₄, 98%, mol.wt. 98.07] slowly and with stirring to about 150mls ultra-pure deionised water in a 500ml graduated beaker. Allow to cool before diluting to the 200ml graduation mark with ultra-pure deionised water. Stable in a refrigerator for six months.

- 4.2 Sulphuric acid (~2.5M). In a fume cupboard, carefully, and with stirring, add 140mls concentrated sulphuric acid [H₂SO₄, 98%, mol.wt. 98.07] to about 800mls ultra-pure deionised water in a one litre graduated beaker. Allow to cool before diluting to the 1000ml graduation mark with ultra-pure deionised water. Stable in a refrigerator for six months.
- 4.3 Sodium hydroxide (~1M). Dissolve 4 ± 0.1 g AR sodium hydroxide [NaOH, mol. wt. 40.0] in about 50mls ultra-pure deionised water in a 100ml graduated beaker and make up to the 100ml graduation mark with ultra-pure deionised water. Prepare fresh as required.
- 4.4 Ammonium molybdate solution. Dissolve 4 ± 0.002g ammonium molybdate [(NH₄)₆Mo₇O₂₄.4H₂O, mol.wt. 1235.9] in about 50mls ultra-pure deionised water in a 100ml graduated beaker and make up to the 100ml graduation mark with ultra-pure deionised water. Stable in a refrigerator for one week.
- 4.5 Antimony potassium tartrate solution. Dissolve 0.340 ± 0.002g antimony (III) potassium oxitartrate trihydrate [C₄H₄KO₇Sb.3H₂O, mol.wt. 378.98], in about 50mls ultra-pure deionised water in a 100ml graduated beaker and make up to the 100ml graduation mark with ultra-pure deionised water. Stable in a refrigerator for one week.
- 4.6 **PhosphatR1 (Aquakem Reagent PHOS 1)**. From a measuring cylinder add 75mls ammonium molybdate solution to 250mls 2.5M sulphuric acid in a beaker and pipette in 25 ± 0.03mls antimony potassium tartrate solution.
- 4.7 **PhosphatR2 (Aquakem Reagent PHOS 2)**. Dissolve 1.76 ± 0.002g L-ascorbic acid [C₆H₈O₆, mol.wt. 176.13] in about 50mls ultra-pure deionised water in a 100ml graduated beaker and make up to the 100ml graduation mark with ultra-pure deionised water. Stable in a refrigerator for five days.
- 4.8 **Na₂HCO₃**. Olsen's reagent. Dissolve 210 ± 0.1 g AR sodium hydrogen carbonate [NaHCO₃, mol. wt. 84.01] in about 1500mls of ultra-pure deionised water in a 2 litre graduated beaker. Add about 100 ml of 1M NaOH to adjust the pH to approximately 8.5 ± 0.05 and transfer quantitatively to a 5 litre volumetric flask. Make up to the mark with ultra-pure deionised water. Stable for 3 months but check/adjust pH before use.
- 4.9 Preparation of calibration standards.
- 4.9.1 Calibration stock solution (1000 mg P / litre). Dissolve 2.197 ± 0.002 g AR potassium dihydrogen orthophosphate [KH₂PO₄, mol. wt. 136.09] in ultra-pure deionised water in a 500ml volumetric flask and make up to the mark with ultra-pure deionised water. Stable in a refrigerator for up to 6 months.
- 4.9.2 Working stock (10 mg P / litre). Dilute 5 ± 0.015 ml stock phosphate solution to 500 ± 0.5 ml with Olsen's reagent.
- 4.9.3 Prepare individual calibration standards as follows:

Calibration standards	
0 mgPO ₄ _P/litre	Olsen's reagent
2 mgPO ₄ _P/litre	Dilute 5mls working stock to 25 ± 0.03mls with Olsen's reagent.
4 mgPO ₄ _P/litre	Dilute 10mls working stock to 25 ± 0.03mls with Olsen's reagent.
6 mgPO ₄ _P/litre	Dilute 15mls working stock to 25 ± 0.03mls with Olsen's reagent.
8 mgPO ₄ _P/litre	Dilute 20mls working stock to 25 ± 0.03mls with Olsen's reagent.

5 PROCEDURES

Soil is normally dried at 25 ± 10 °C to constant weight or overnight and ground to pass a 2 mm sieve. Dried soil samples may be stored at room temperature. Fresh soil samples, if not dried immediately, should be stored at <10 °C in a refrigerator.

5.1 Preparation of Soil Extract. *Include at least one blank extraction.*

5.1.1 Weigh accurately about 2.5 g dried soil into a wide necked polyethylene bottle (maximum volume 250 ml) and add 50 ± 0.1 ml Olsen's reagent. Larger weights of soil may be extracted, provided that a soil/extractant ratio of 1:20 is used and there is sufficient headspace to allow complete extraction (see Appendix 3).

5.1.2 Cap the bottle and place on an orbital shaker for 30 ± 5 minutes at 20 ± 2 °C.

5.1.3 Filter the extract through Whatman No 2 filter paper into a suitable sample tube. Extracts can be stored in a refrigerator overnight. (Laboratory /Field Notebook No. 00004, pp136-138).

5.1.4 Pipette 2.5 ± 0.01 mls extract or calibration standard into a suitable vial (~20mls to allow for frothing, slowly add 0.5 ± 0.05 mls of 1.5M sulphuric acid and gently swirl to release carbon dioxide.

5.2 **Analysis.** For setting up and running the analyser, refer to equipment operating procedure EOP 219. The acidified extracts are transferred to polystyrene sample cups and loaded into sample segments in the normal way.

5.3 **Calculation of results. (Only applies where 2.5mls extract is taken for analysis).**

$$\text{Extractable P (mg/kg soil)} = \frac{(\text{mg P/litre in sample} - \text{blank}) \times 50}{\text{weight soil (g)}}$$

APPENDIX 1 - METHOD VALIDATION

Data source	Assessment	mg extractable PO ₄ _P/litre
Validation folder	Limit of detection (LOD)	No data
Validation folder	Limit of quantification (LOQ)	No data
Validation folder	Working range	No data
AQC and Aquacheck data	Accuracy/Bias	No data
Validation folder	Within-run precision (RSD%)	No data
AQC/XXX & YYY	Within-lab precision (RSD%)	No data
Uncertainty file	Uncertainty - 95% Confidence limit	No data

APPENDIX 2 - BLANK VALUES AND PURITY OF DEIONISED WATER

Average blank values from either Elga reverse osmosis water or Milli-Q ultra pure water were:

Elga reverse osmosis water- 29.442 µgPO₄_P/litre (N = 28), rel. std. deviation 3.2%
 Milli-Q ultra pure water - 7.093 µgPO₄_P/litre (N = 28), rel. std. deviation 8.3%

APPENDIX 3 – INFLUENCE OF METHOD OF SHAKING ON EXTRACTABLE PHOSPHATE

Extraction method					Mean values for Olsen P (mg/kg dry soil) (S.E. mean in italics)	
Soil (g)	Extractant (mls)	Shaker	Duration (minutes)	Bottle position	Soil 1	AQC/39
2.5	50	Orbital	60	Upright	27.6 (<i>0.23</i>)	135
20	400	Orbital	60	Upright	9.06 (<i>0.32</i>)	38.4
20	400	Orbital	60	On side	24.3 (<i>0.40</i>)	122

The 2.5:50 extract had more space in the bottle than the 20:400 extracts; thus the most phosphate was extracted (Data from Rachel Matthews, November 2010).

Appendices

APPENDIX 4 – TEST PARAMETERS

Name of test	Phosphate				
Online name	Olsen P	Test in Use	YES		
Test type	Photometric		LOW		HIGH
Results unit	mg/l	Test limit	0		24 mg/l
Number of decimals	3	Initial absorbance	*	*A	
		Dilution limit	*	8 mg/l	
		Secondary dilution 1+	0.0	2.0	
		Critical limit	*	* mg/l	
		Reflex test limit	*	* mg/l	
		Reflex test			
Acceptance	Manual	Reference class	LOW	HIGH	In use
Dilution 1+	0.0				
Sample type	Bicarbonate extract	Correction factor	1.00		
Calibration type	Polynomial				
Curve direction	Ascending				
Repeat time (d)	0	Abs error (mA)	*		
Points/cal.	Single	rel error (%)			
Acceptance	Manual				
Response limit (mA)	MIN	MAX			
	*	*			
Coeff. Of determination	0.99500				
Bias correction in use	NO				
Cd reduction	NO				
Type of Calibrators	Separate				
Calibrator	Conc.	Dil. Ratio			
Olsen P-0	0.000	1+0.0			
Olsen - 2	2.000	1+0.0			
Olsen - 4	4.000	1+0.0			
Olsen - 6	6.000	1+0.0			
Olsen - 8	8.000	1+0.0			
Manual QC in Use	NO	Routine QC in use	NO		
Blank	YES				
		Normal cuvette			
Sample		Volume (µl)	100		
Disp. With	Extra	Add. Volume (µl)	40		
Dilution with	Special	Wash reagent	None		
Raw sample					
Disp. with	Extra	Add Volume (µl)	50		
Special diluent	Na2HCO3				
Disp. with	Extra	Add Volume (µl)	20		
Syringe speed	Normal				
Measurement	End point	Blank			
Resp. Min (A)	*	Resp. Max (A)	*		
Reagent	PhosphatR1	Volume (µl)	40		
Disp. with	Extra	Add. Volume (µl)	50		
Wash reagent	None				
Syringe speed	Normal				
Additional mixing					
Reagent	PhosphatR2	Volume (µl)	20		
Disp. with	Water	Add. Volume (µl)	30		
Wash reagent	None				
Syringe speed	Normal				
Incubation	Time (sec)	540			
Measurement	End point				
Wavelength (nm)	880nm	Side wavel. (nm)	None		
Meas. type	Fixed timing				

Appendix C

Chapter IV

- C1. Pictures
- C2. Tables
- C3. Figures

C1. Pictures



Picture C1-1. The completed frame, lighting system and all 48 pots, before the soil was added.

C2. Tables

Treatments		ER			GPP		
		t	df	Sig. (2-tailed)	t	df	Sig. (2-tailed)
Pair 1	Control_Oct12 - Warmed_Oct12	.046	23	.964	.225	23	.824
Pair 2	Control_Nov12 - Warmed_Nov12	-.341	23	.736	-.534	23	.598
Pair 3	Control_Dec12 - Warmed_Dec12	-3.631	23	.001	-1.605	23	.122
Pair 4	Control_Jan13 - Warmed_Jan13	-3.959	23	.001	-1.771	23	.090
Pair 5	Control_Feb13 - Warmed_Feb13	-1.177	23	.251	-.597	23	.556
Pair 6	Control_March13 - Warmed_March13	-.754	23	.458	-.528	23	.603
Pair 7	Control_April13 - Warmed_April13	.708	23	.486	1.196	23	.244
Pair 8	Control_May13 - Warmed_May13	.927	23	.364	.855	23	.401
Pair 9	Control_June13 - Warmed_June13	.636	23	.531	.419	23	.679
Pair 10	Control_July13 - Warmed_July13	.420	23	.678	.678	23	.505

Table C2-1. Pairwise t-test results for ecosystem respiration (ER) and gross primary productivity (GPP) comparisons between control and warmed treatments

C3. Figures

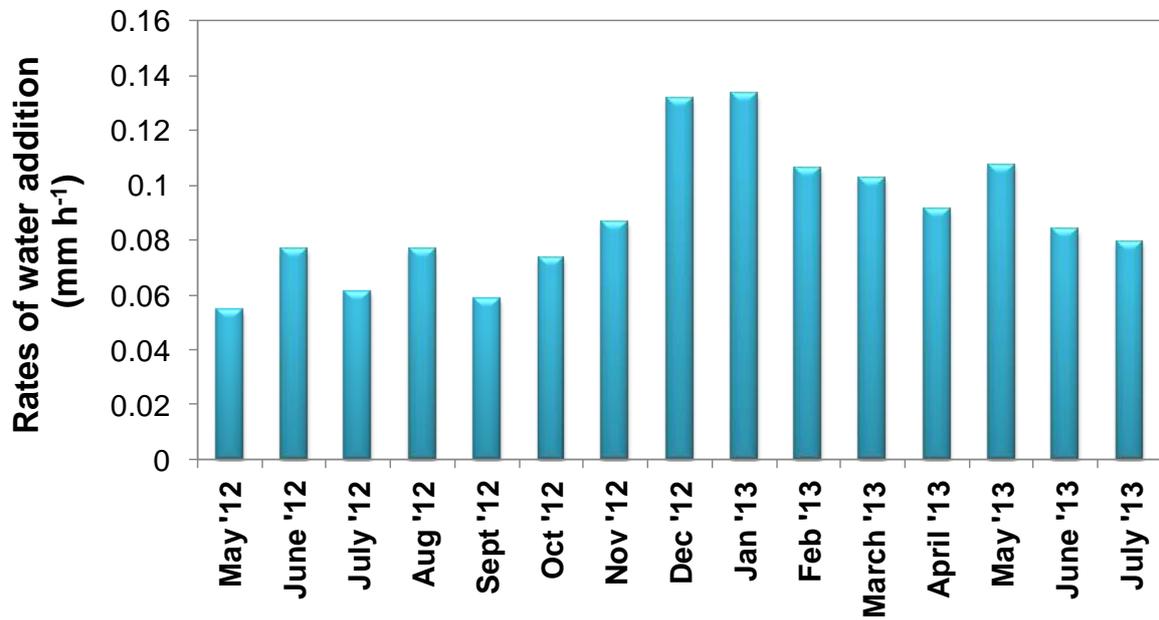


Figure C3-1. Rates of monthly water additions to each lysimeter during the entire experiment

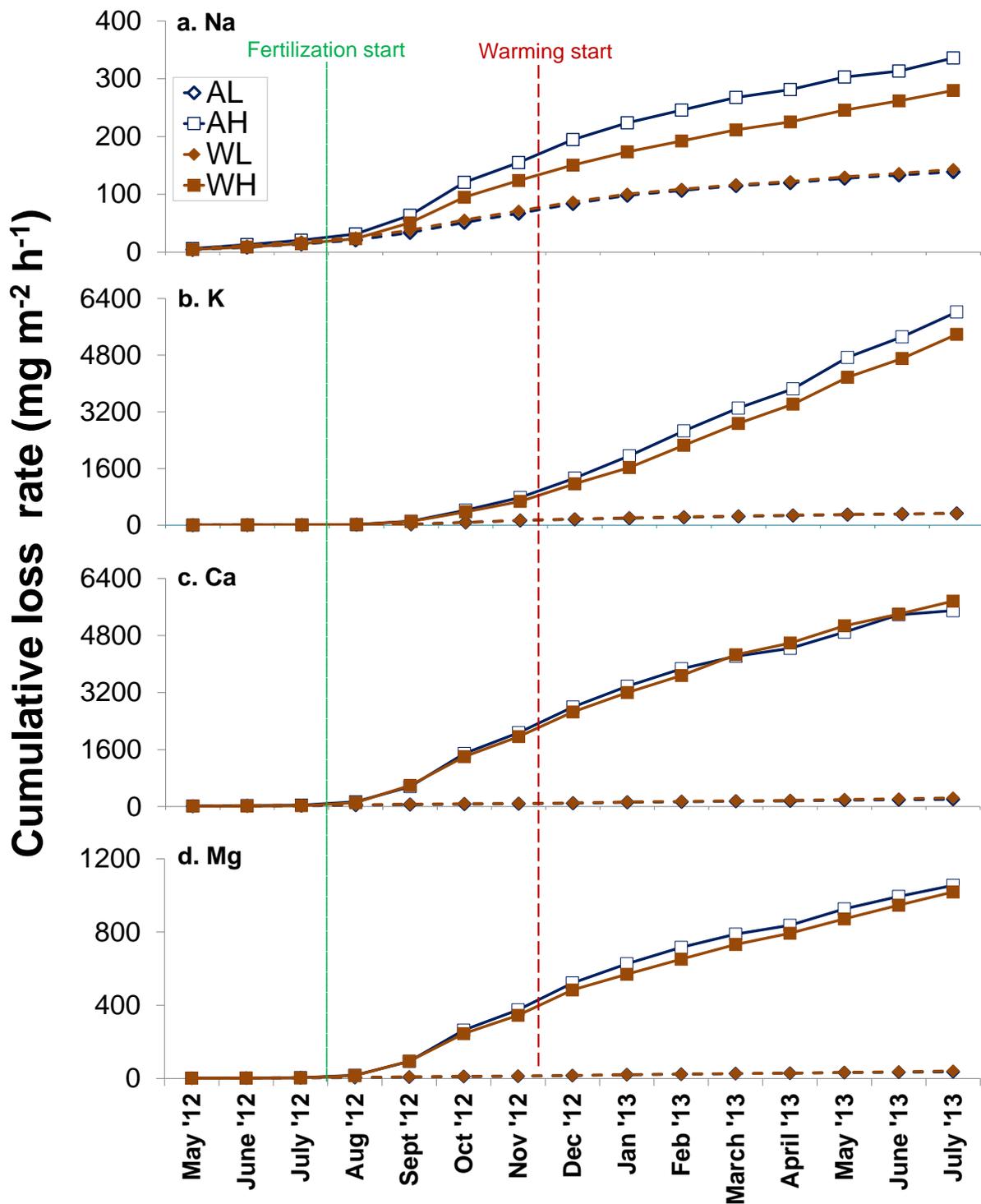


Figure C3-2. Cumulative cations loss in leachate from soil only lysimeters, relative to the main treatments applied to the lysimeters: nutrient additions (continuous vertical line) and warming (dashed vertical line). The panels indicate the four main cations: sodium – Na⁺ (a.), potassium – K⁺ (b.), calcium – Ca²⁺ (c.) and magnesium – Mg²⁺ (d.). Error bars are ±SE (n=4).

Appendices

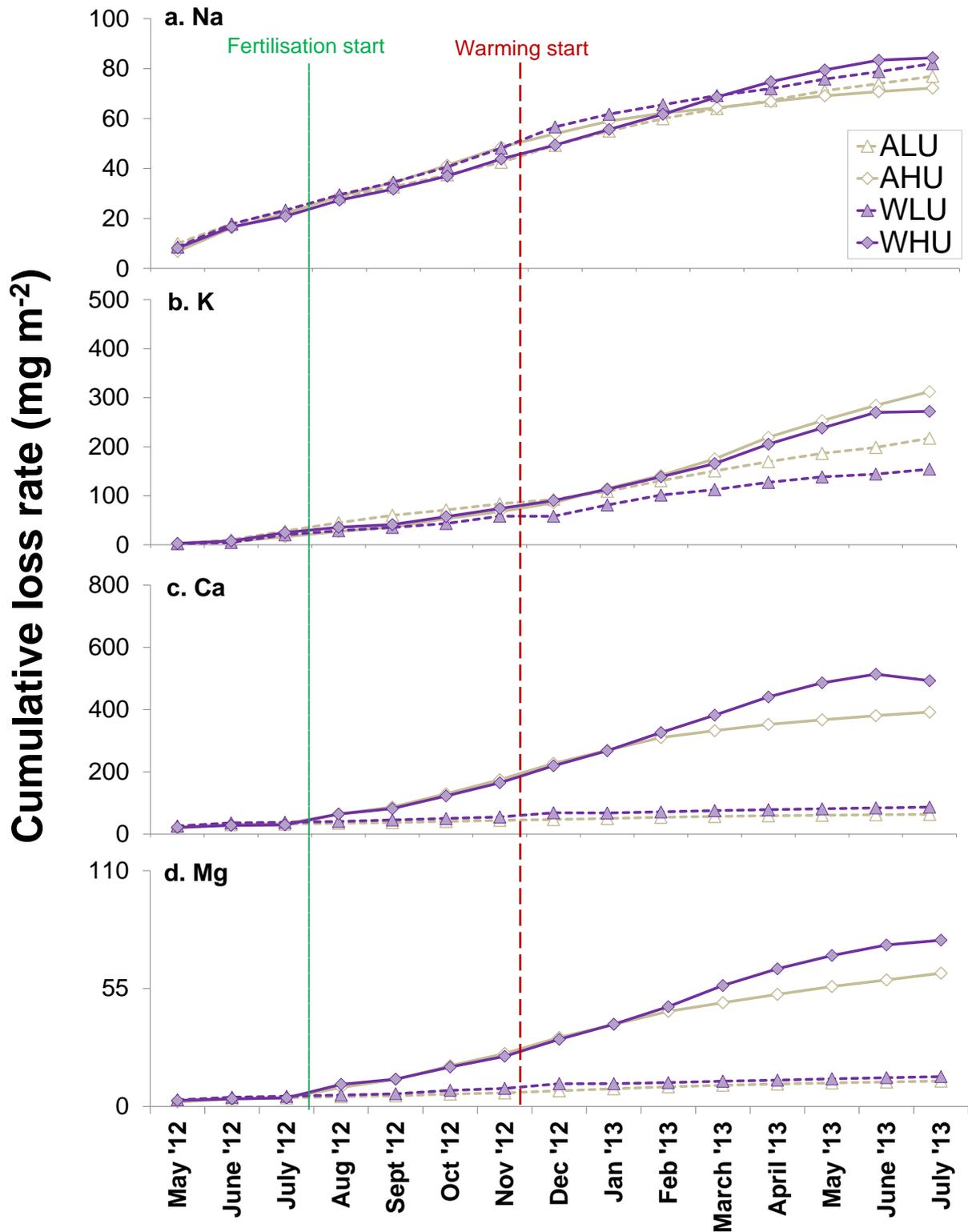


Figure C3-3. Cumulative cations loss rates in leachate from unclipped planted lysimeters, relative to the main treatments applied to the pots: nutrient additions (continuous vertical line) and warming (dashed vertical line). The panels indicate the four main cations: sodium – Na⁺ (a.), potassium – K⁺ (b.), calcium – Ca²⁺ (c.) and magnesium – Mg²⁺ (d.). Error bars are ±SE (n=4).

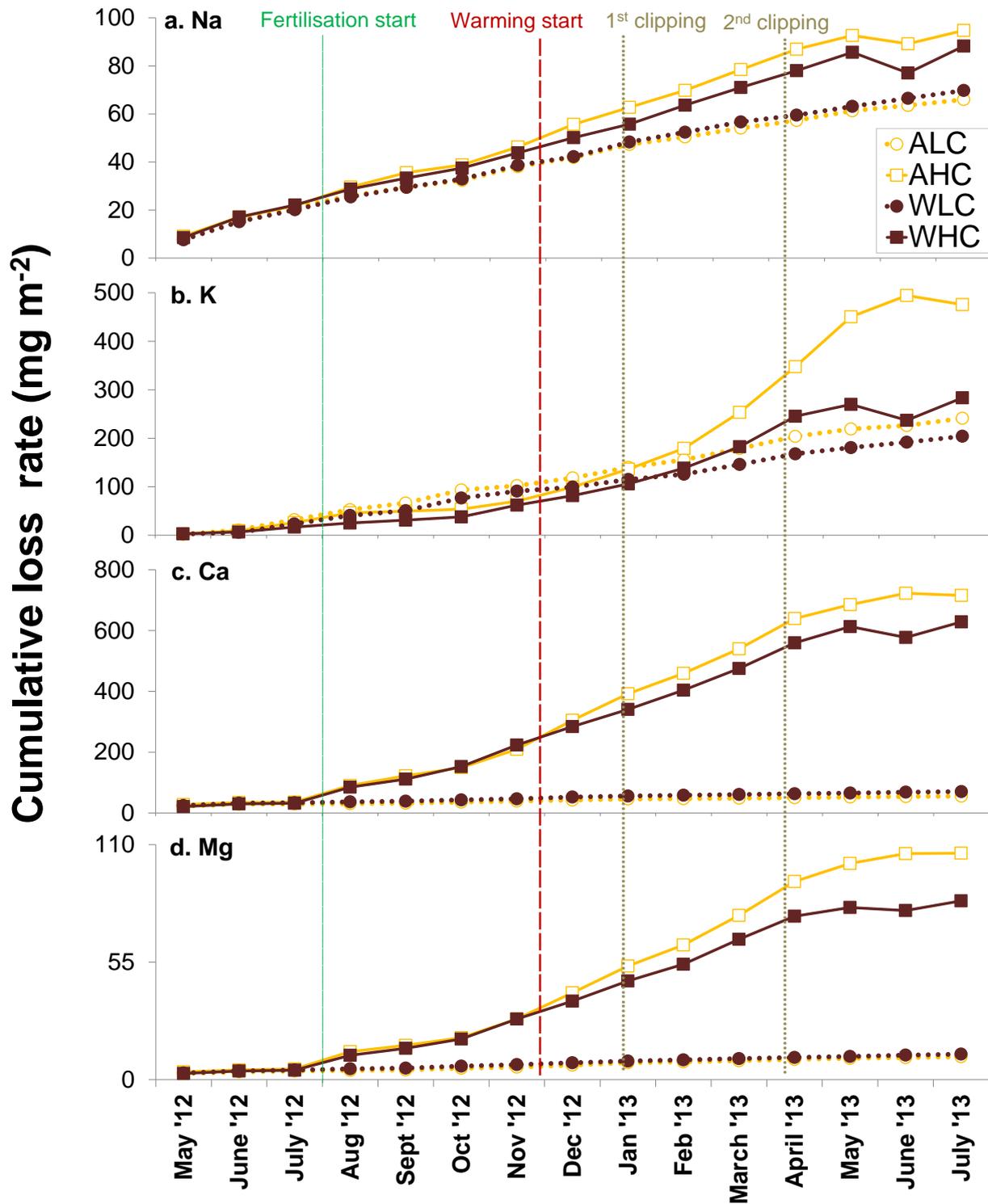


Figure C3-4. Cumulative cations loss rates in leachate from clipped planted lysimeters, relative to the main treatments applied to the lysimeters: nutrient additions (continuous vertical line), warming (dashed vertical line) and clipping (dotter vertical line). The panels indicate the four main cations: sodium – Na⁺ (a.), potassium – K⁺ (b.), calcium – Ca²⁺ (c.) and magnesium – Mg²⁺ (d.). Error bars are ±SE (n=4).

Appendices

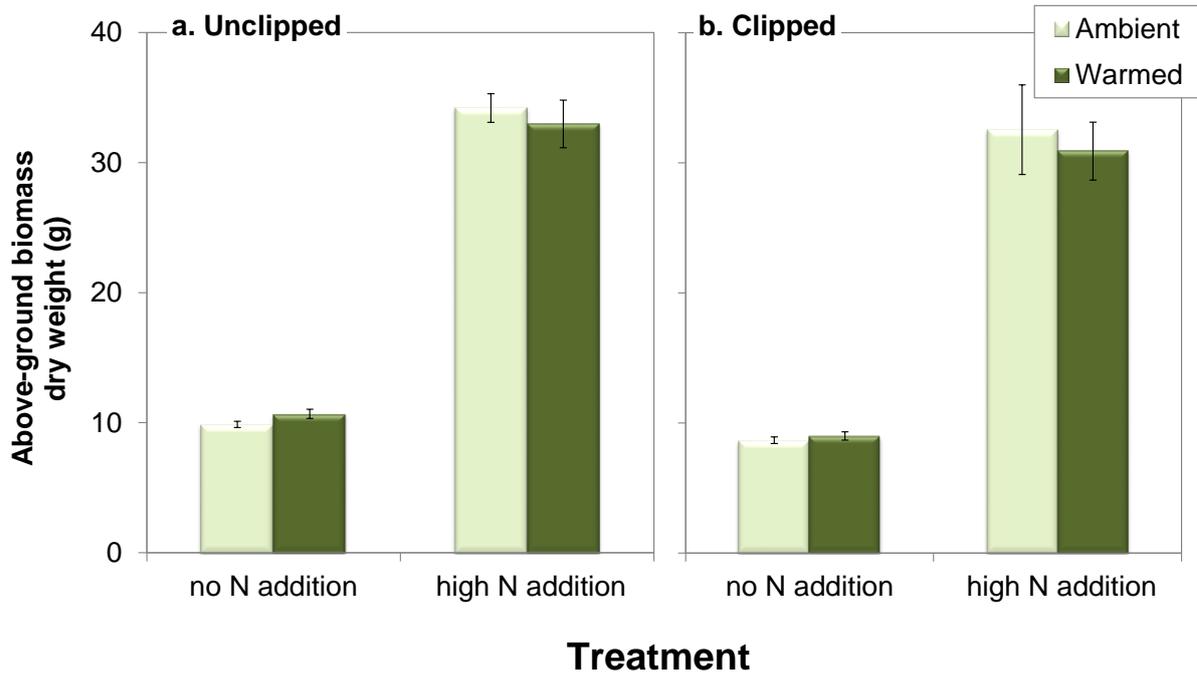


Figure C3-5. Dry weight of the above-ground biomass, after the completion of the experiment

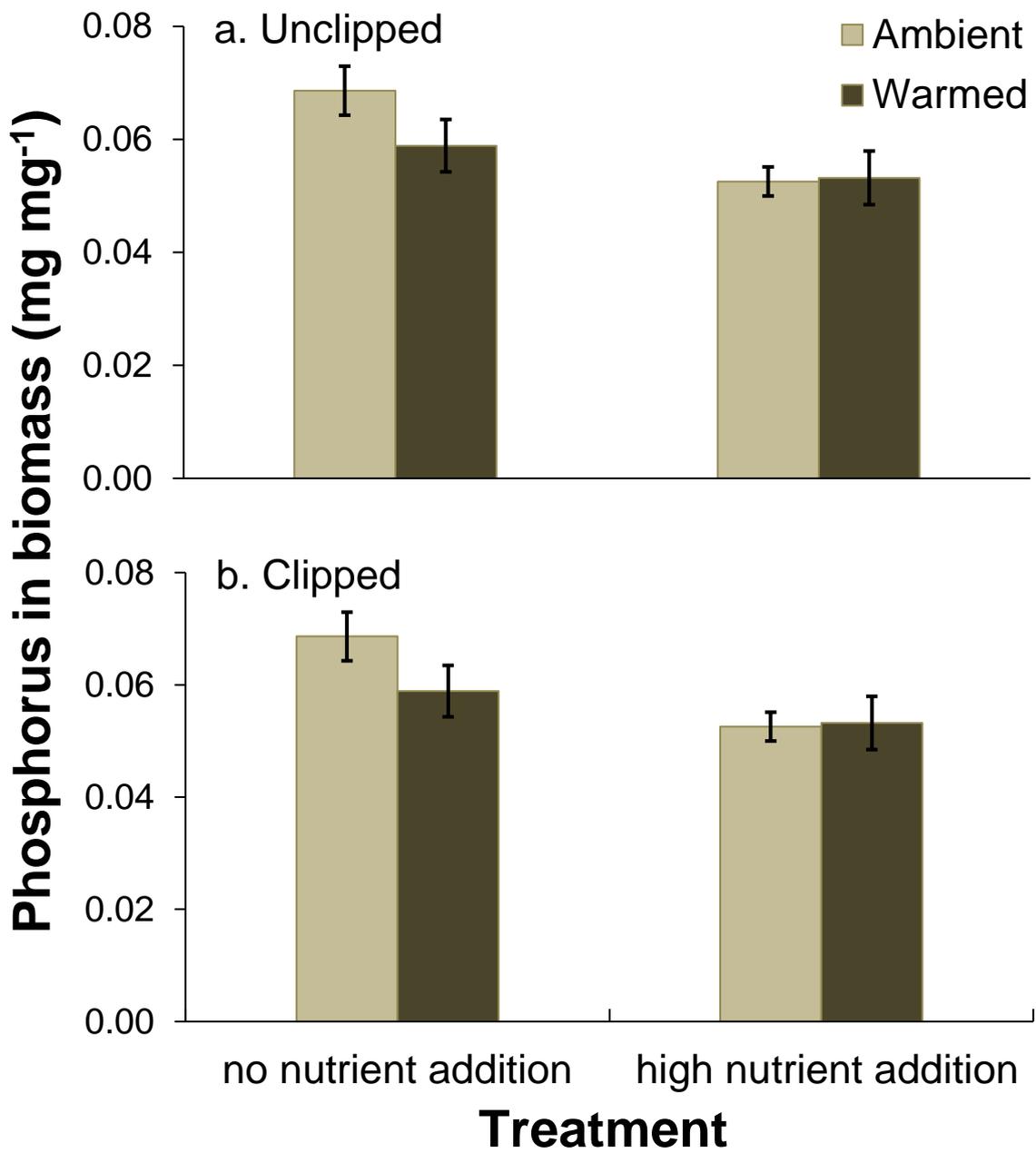


Figure C3-6. P concentration in above ground biomass, calculated for each lysimeter

7. REFERENCES

- ABER, J., MCDOWELL, W., NADELHOFFER, K., MAGILL, A., BERNTSON, G., KAMAKEA, M., MCNULTY, S., CURRIE, W., RUSTAD, L. & FERNANDEZ, I. 1998. Nitrogen Saturation in Temperate Forest Ecosystems. *BioScience*, 921-934.
- ABER, J. D. 1992. Nitrogen Cycling and Nitrogen Saturation in Temperate Forest Ecosystems. *Trends in Ecology & Evolution*, 7, 220-224.
- AINSWORTH, E. A. & LONG, S. P. 2005. What Have We Learned from 15 Years of Free-Air CO₂ Enrichment (FACE)? A Meta-Analytic Review of the Responses of Photosynthesis, Canopy. *New Phytologist*, 165, 351-371.
- ALLEN, S. E., GRIMSHAW, H., PARKINSON, J. A. & QUARMBY, C.-I. 1974. *Chemical Analysis of Ecological Materials*, Blackwell Scientific Publications.
- ANDERSON, J. 1992. Responses of Soils to Climate Change. *Advances in ecological research*, 22, 163-210.
- ARNETH, A., HARRISON, S. P., ZAEHLE, S., TSIGARIDIS, K., MENON, S., BARTLEIN, P. J., FEICHTER, J., KORHOLA, A., KULMALA, M., O'DONNELL, D., SCHURGERS, G., SORVARI, S. & VESALA, T. 2010. Terrestrial Biogeochemical Feedbacks in the Climate System. *Nature Geoscience*, 3, 525-532.
- ARRONIZ-CRESPO, M., LEAKE, J. R., HORTON, P. & PHOENIX, G. K. 2008. Bryophyte Physiological Responses to, and Recovery from, Long-Term Nitrogen Deposition and Phosphorus Fertilisation in Acidic Grassland. *New Phytologist*, 180, 864-874.
- AUSTIN, A. T. & BALLARÉ, C. L. 2010. Dual Role of Lignin in Plant Litter Decomposition in Terrestrial Ecosystems. *Proceedings of the National Academy of Sciences*, 107, 4618-4622.
- AVERILL, C., TURNER, B. L. & FINZI, A. C. 2014. Mycorrhiza-Mediated Competition between Plants and Decomposers Drives Soil Carbon Storage. *Nature*, 505, 543-545.
- BADER, D., COVEY, C., GUTOWSKI, W., HELD, I., KUNKEL, K., MILLER, R., TOKMAKIAN, R. & ZHANG, M. 2008. Climate Models: An Assessment of Strengths and Limitations. *US Department of Energy Publications*, 8.
- BAHN, M., REICHSTEIN, M., DAVIDSON, E., GRUNZWEIG, J., JUNG, M., CARBONE, M., EPRON, D., MISSON, L., NOUVELLON, Y. & ROUPSARD, O. 2010. Soil Respiration at Mean Annual Temperature Predicts Annual Total across Vegetation Types and Biomes. *Biogeosciences*.
- BALIGAR, V., FAGERIA, N. & HE, Z. 2001. Nutrient Use Efficiency in Plants. *Communications in Soil Science and Plant Analysis*, 32, 921-950.
- BEER, C., REICHSTEIN, M., TOMELLERI, E., CIAIS, P., JUNG, M., CARVALHAIS, N., RÖDENBECK, C., ARAIN, M. A., BALDOCCHI, D. & BONAN, G. B. 2010. Terrestrial Gross Carbon Dioxide Uptake: Global Distribution and Covariation with Climate. *Science*, 329, 834-838.
- BELLAMY, P. H., LOVELAND, P. J., BRADLEY, R. I., LARK, R. M. & KIRK, G. J. D. 2005. Carbon Losses from All Soils across England and Wales 1978–2003. *Nature*, 437, 245-248.
- BENDER, M. 1968. Mass Spectrometry Studies of Carbon 13 Variations in Corn and Other Grasses. *Radiocarbon*, 10, 468-472.

- BERNHARD, A. 2012. The Nitrogen Cycle: Processes, Players, and Human Impact. *Nature Education Knowledge*, 3, 25.
- BILOTTA, G., BRAZIER, R. & HAYGARTH, P. 2007. The Impacts of Grazing Animals on the Quality of Soils, Vegetation, and Surface Waters in Intensively Managed Grasslands. *Advances in Agronomy*, 94, 237-280.
- BINKLEY, D., SENOCK, R. & CROMACK, K. 2003. Phosphorus Limitation on Nitrogen Fixation by *Facaltaria* Seedlings. *Forest Ecology and Management*, 186, 171-176.
- BOBBINK, R. & HETTELINGH, J.-P. Review and Revision of Empirical Critical Loads and Dose-Response Relationships. Proceedings of an expert workshop, Noordwijkerhout, 2010. 23-25.
- BOBBINK, R., HICKS, K., GALLOWAY, J., SPRANGER, T., ALKEMADE, R., ASHMORE, M., BUSTAMANTE, M., CINDERBY, S., DAVIDSON, E., DENTENER, F., EMMETT, B., ERISMAN, J. W., FENN, M., GILLIAM, F., NORDIN, A., PARDO, L. & DE VRIES, W. 2010. Global Assessment of Nitrogen Deposition Effects on Terrestrial Plant Diversity: A Synthesis. *Ecological Applications*, 20, 30-59.
- BOBBINK, R., HORNING, M. & ROELOFS, J. G. 1998. The Effects of Air-Borne Nitrogen Pollutants on Species Diversity in Natural and Semi-Natural European Vegetation. *Journal of Ecology*, 86, 717-738.
- BOBBINK, R. & ROELOFS, J. G. M. 1995. Nitrogen Critical Loads for Natural and Semi-Natural Ecosystems: The Empirical Approach. *Water, Air, & Soil Pollution*, 85, 2413-2418.
- BONAN, G. B. & LEVIS, S. 2010. Quantifying Carbon-Nitrogen Feedbacks in the Community Land Model (Cm4). *Geophysical Research Letters*, 37, L07401.
- BOND-LAMBERTY, B. & THOMSON, A. 2010a. A Global Database of Soil Respiration Data. *Biogeosciences*, 7, 1915-1926.
- BOND-LAMBERTY, B. & THOMSON, A. 2010b. Temperature-Associated Increases in the Global Soil Respiration Record. *Nature*, 464, 579-U132.
- BOWDEN, R. D., DAVIDSON, E., SAVAGE, K., ARABIA, C. & STEUDLER, P. 2004. Chronic Nitrogen Additions Reduce Total Soil Respiration and Microbial Respiration in Temperate Forest Soils at the Harvard Forest. *Forest Ecology and Management*, 196, 43-56.
- BRADFORD, M. A., FIERER, N., JACKSON, R. B., MADDOX, T. R. & REYNOLDS, J. F. 2008. Nonlinear Root-Derived Carbon Sequestration across a Gradient of Nitrogen and Phosphorous Deposition in Experimental Mesocosms. *Global Change Biology*, 14, 1113-1124.
- BRADFORD, M. A., WARREN II, R. J., BALDRIAN, P., CROWTHER, T. W., MAYNARD, D. S., OLDFIELD, E. E., WIEDER, W. R., WOOD, S. A. & KING, J. R. 2014. Climate Fails to Predict Wood Decomposition at Regional Scales. *Nature Clim. Change*, 4, 625-630.
- BRAGAZZA, L., FREEMAN, C., JONES, T., RYDIN, H., LIMPENS, J., FENNER, N., ELLIS, T., GERDOL, R., HAJEK, M., HAJEK, T., LACUMIN, P., KUTNAR, L., TAHVANAINEN, T. & TOBERMAN, H. 2006. Atmospheric Nitrogen Deposition Promotes Carbon Loss from Peat Bogs. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 19386-19389.
- BRAZIER, R., TURNBULL, L., WAINWRIGHT, J. & BOL, R. 2014. Carbon Loss by Water Erosion in Drylands: Implications from a Study of Vegetation Change in the South-West USA. *Hydrological Processes*, 28, 2212-2222.

- BUCHER, M. 2007. Functional Biology of Plant Phosphate Uptake at Root and Mycorrhiza Interfaces. *New Phytologist*, 173, 11-26.
- BULLOCK, J. M., JEFFERSON, R. G., BLACKSTOCK, T. H., PAKEMAN, R. J., EMMETT, B. A., PYWELL, R. J., GRIME, J. P. & SILVERTOWN, J. 2011. Semi-Natural Grasslands (In: Technical Report - the UK National Ecosystem Assessment). *Cambridge, UK, UNEP-WCMC*, pp162-195.
- BURTON, A. J., PREGITZER, K. S., CRAWFORD, J. N., ZOGG, G. P. & ZAK, D. R. 2004. Simulated Chronic NO₃⁻ Deposition Reduces Soil Respiration in Northern Hardwood Forests. *Global Change Biology*, 10, 1080-1091.
- BUTTERBACH-BAHL, K., GUNDERSEN, P., AMBUS, P., AUGUSTIN, J., BEIER, C., BOECKX, P., DANNENMANN, M., SANCHEZ GIMENO, B., IBROM, A. & KIESE, R. 2011. Nitrogen Processes in Terrestrial Ecosystems. *The European nitrogen assessment: sources, effects and policy perspectives*, 99-125.
- BYRNE, K. A., KIELY, G. & LEAHY, P. 2005. CO₂ Fluxes in Adjacent New and Permanent Temperate Grasslands. *Agricultural and Forest Meteorology*, 135, 82-92.
- CAMPBELL, N. A. & REECE, J. B. 2011. *Biology*, Pearson.
- CARROLL, J. A., CAPORN, S. J. M., JOHNSON, D., MORECROFT, M. D. & LEE, J. A. 2003. The Interactions between Plant Growth, Vegetation Structure and Soil Processes in Semi-Natural Acidic and Calcareous Grasslands Receiving Long-Term Inputs of Simulated Pollutant Nitrogen Deposition. *Environmental Pollution*, 121, 363-376.
- CHAPIN III, F. S., CHAPIN, M. C., MATSON, P. A. & VITOUSEK, P. 2011. *Principles of Terrestrial Ecosystem Ecology*, Springer.
- CHENG, X., LUO, Y., XU, X., SHERRY, R. & ZHANG, Q. 2011. Soil Organic Matter Dynamics in a North America Tallgrass Prairie after 9 Yr of Experimental Warming. *Biogeosciences*, 8, 1487-1498.
- CLELAND, E. E. & HARPOLE, W. S. 2010. Nitrogen Enrichment and Plant Communities. *Annals of the New York Academy of Sciences*, 1195, 46-61.
- CLEVELAND, C. C., TOWNSEND, A. R., SCHIMEL, D. S., FISHER, H., HOWARTH, R. W., HEDIN, L. O., PERAKIS, S. S., LATTY, E. F., VON FISCHER, J. C. & ELSEROD, A. 1999. Global Patterns of Terrestrial Biological Nitrogen (N₂) Fixation in Natural Ecosystems. *Global Biogeochemical Cycles*, 13, 623-645.
- CONANT, R. T., PAUSTIAN, K. & ELLIOTT, E. T. 2001. Grassland Management and Conversion into Grassland: Effects on Soil Carbon. *Ecological Applications*, 11, 343-355.
- COOK, B. D. & ALLAN, D. L. 1992. Dissolved Organic Carbon in Old Field Soils: Total Amounts as a Measure of Available Resources for Soil Mineralization. *Soil Biology and Biochemistry*, 24, 585-594.
- COSBY, B. J., FERRIER, R. C., JENKINS, A. & WRIGHT, R. F. 2001. Modelling the Effects of Acid Deposition: Refinements, Adjustments and Inclusion of Nitrogen Dynamics in the MAGIC Model. *Hydrology and Earth System Sciences Discussions*, 5, 499-518.
- CRAINE, J., MORROW, C. & FIERER, N. 2007. Microbial Nitrogen Limitation Increases Decomposition. *Ecology*, 88, 2105-2113.
- CRAWFORD, M., GRACE, P., BELLOTTI, W. & OADES, J. Below-Ground Inputs of Carbon by Crops and Pastures. *Proceedings of the 8th Australian Agronomy Conference Queensland, Australia, 1996*. 172-175.

- CREWS, T. E. & PEOPLES, M. B. 2004. Legume Versus Fertilizer Sources of Nitrogen: Ecological Tradeoffs and Human Needs. *Agriculture, Ecosystems & Environment*, 102, 279-297.
- CUSACK, D. F., SILVER, W. L., TORN, M. S. & MCDOWELL, W. H. 2011. Effects of Nitrogen Additions on above- and Belowground Carbon Dynamics in Two Tropical Forests. *Biogeochemistry*, 104, 203-225.
- DAVIDSON, E., DAVID, M. B., GALLOWAY, J. N., GOODALE, C. L., HAEUBER, R., HARRISON, J. A., HOWARTH, R. W., JAYNES, D. B., LOWRANCE, R. R. & NOLAN, B. T. 2012. Excess Nitrogen in the U. S. Environment: Trends, Risks, and Solutions. *Issues in Ecology*.
- DAVIDSON, E. A. & JANSSENS, I. A. 2006. Temperature Sensitivity of Soil Carbon Decomposition and Feedbacks to Climate Change. *Nature*, 440, 165-173.
- DAVIDSON, E. A., TRUMBORE, S. E. & AMUNDSON, R. 2000. Biogeochemistry: Soil Warming and Organic Carbon Content. *Nature*, 408, 789-790.
- DE VALPINE, P. & HARTE, J. 2001. Plant Responses to Experimental Warming in a Montane Meadow. *Ecology*, 82, 637-648.
- DE VRIES, W., REINDS, G. J., GUNDERSEN, P. E. R. & STERBA, H. 2006. The Impact of Nitrogen Deposition on Carbon Sequestration in European Forests and Forest Soils. *Global Change Biology*, 12, 1151-1173.
- DE VRIES, W., SOLBERG, S., DOBBERTIN, M., STERBA, H., LAUBHANN, D., VAN OIJEN, M., EVANS, C., GUNDERSEN, P., KROS, J., WAMELINK, G. W. W., REINDS, G. J. & SUTTON, M. A. 2009. The Impact of Nitrogen Deposition on Carbon Sequestration by European Forests and Heathlands. *Forest Ecology and Management*, 258, 1814-1823.
- DEL GALDO, I., SIX, J., PERESSOTTI, A. & COTRUFO, M. F. 2003. Assessing the Impact of Land-Use Change on Soil C Sequestration in Agricultural Soils by Means of Organic Matter Fractionation and Stable C Isotopes. *Global Change Biology*, 9, 1204-1213.
- DELUCIA, E. H., MOORE, D. J. & NORBY, R. J. 2005. Contrasting Responses of Forest Ecosystems to Rising Atmospheric Co₂: Implications for the Global C Cycle. *Global Biogeochemical Cycles*, 19, 9.
- DIJKSTRA, F. A., CHENG, W. & JOHNSON, D. W. 2006. Plant Biomass Influences Rhizosphere Priming Effects on Soil Organic Matter Decomposition in Two Differently Managed Soils. *Soil Biology and Biochemistry*, 38, 2519-2526.
- DISE, N. B. & STEVENS, J. 2005. Nitrogen Deposition and Reduction of Terrestrial Biodiversity: Evidence from Temperate Grasslands. *Science in China Series C: Life Sciences*, 48, 720-728.
- DRINKWATER, L. E., WAGONER, P. & SARRANTONIO, M. 1998. Legume-Based Cropping Systems Have Reduced Carbon and Nitrogen Losses. *Nature*, 396, 262-265.
- DUKES, J. S., CHIARIELLO, N. R., CLELAND, E. E., MOORE, L. A., SHAW, M. R., THAYER, S., TOBECK, T., MOONEY, H. A. & FIELD, C. B. 2005. Responses of Grassland Production to Single and Multiple Global Environmental Changes. *Plant Biology*, 3, 1829-1837.
- DUNGAIT, J. A., CARDENAS, L. M., BLACKWELL, M. S., WU, L., WITHERS, P. J., CHADWICK, D. R., BOL, R., MURRAY, P. J., MACDONALD, A. J. & WHITMORE, A. P. 2012. Advances in the Understanding of Nutrient Dynamics and Management in Uk Agriculture. *Science of the Total Environment*, 434, 39-50.

- DUNGAIT, J. A. J., KEMMITT, S. J., MICHALLON, L., GUO, S., WEN, Q., BROOKES, P. C. & EVERSLED, R. P. 2011. Variable Responses of the Soil Microbial Biomass to Trace Concentrations of ¹³C-Labelled Glucose, Using ¹³C-Plfa Analysis. *European Journal of Soil Science*, 62, 117-126.
- ELSGAARD, L., GÖRRES, C.-M., HOFFMANN, C. C., BLICHER-MATHIESEN, G., SCHELDE, K. & PETERSEN, S. O. 2012. Net Ecosystem Exchange of Co₂ and Carbon Balance for Eight Temperate Organic Soils under Agricultural Management. *Agriculture, Ecosystems & Environment*, 162, 52-67.
- EVANS, C. D., CAPORN, S. J. M., CARROLL, J. A., PILKINGTON, M. G., WILSON, D. B., RAY, N. & CRESSWELL, N. 2006. Modelling Nitrogen Saturation and Carbon Accumulation in Heathland Soils under Elevated Nitrogen Deposition. *Environmental Pollution*, 143, 468-478.
- FANG, C. & MONCRIEFF, J. 1998. An Open-Top Chamber for Measuring Soil Respiration and the Influence of Pressure Difference on Co₂ Efflux Measurement. *Functional Ecology*, 12, 319-325.
- FILIPPELLI, G. M. 2002. The Global Phosphorus Cycle. *Reviews in mineralogy and geochemistry*, 48, 391-425.
- FILIPPELLI, G. M. 2008. The Global Phosphorus Cycle: Past, Present, and Future. *Elements*, 4, 89-95.
- FONTAINE, S., BARDOUX, G., ABBADIE, L. & MARIOTTI, A. 2004. Carbon Input to Soil May Decrease Soil Carbon Content. *Ecology letters*, 7, 314-320.
- FORNARA, D. A., BANIN, L. & CRAWLEY, M. J. 2013. Multi-Nutrient Vs. Nitrogen-Only Effects on Carbon Sequestration in Grassland Soils. *Global change biology*, 19, 3848-3857.
- FORNARA, D. A. & TILMAN, D. 2008. Plant Functional Composition Influences Rates of Soil Carbon and Nitrogen Accumulation. *Journal of Ecology*, 96, 314-322.
- FORNARA, D. A. & TILMAN, D. 2012. Soil Carbon Sequestration in Prairie Grasslands Increased by Chronic Nitrogen Addition. *Ecology*, 93, 2030-2036.
- FOWLER, D., CAPE, J., UNSWORTH, M., MAYER, H., CROWTHER, J., JARVIS, P., GARDINER, B. & SHUTTLEWORTH, W. 1989. Deposition of Atmospheric Pollutants on Forests [and Discussion]. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 324, 247-265.
- FRIEDLINGSTEIN, P., COX, P., BETTS, R., BOPP, L., VON BLOH, W., BROVKIN, V., CADULE, P., DONEY, S., EBY, M., FUNG, I., BALA, G., JOHN, J., JONES, C., JOOS, F., KATO, T., KAWAMIYA, M., KNORR, W., LINDSAY, K., MATTHEWS, H. D., RADDATZ, T., RAYNER, P., REICK, C., ROECKNER, E., SCHNITZLER, K. G., SCHNUR, R., STRASSMANN, K., WEAVER, A. J., YOSHIKAWA, C. & ZENG, N. 2006. Climate-Carbon Cycle Feedback Analysis: Results from the (Cmip)-M-4 Model Intercomparison. *Journal of Climate*, 19, 3337-3353.
- FRY, B. 2006. *Stable Isotope Ecology*, Springer Verlag.
- GALITZ, D. S. 2009. Uptake and Assimilation of Nitrogen by Plants.
- GALLOWAY, J. N. & COWLING, E. B. 2002. Reactive Nitrogen and the World: 200 Years of Change. *AMBIO: A Journal of the Human Environment*, 31, 64-71.
- GALLOWAY, J. N., DENTENER, F. J., CAPONE, D. G., BOYER, E. W., HOWARTH, R. W., SEITZINGER, S. P., ASNER, G. P., CLEVELAND, C. C., GREEN, P. A., HOLLAND, E. A.,

- KARL, D. M., MICHAELS, A. F., PORTER, J. H., TOWNSEND, A. R. & VÅRÅSMARTY, C. J. 2004. Nitrogen Cycles: Past, Present, and Future. *Biogeochemistry*, 70, 153-226.
- GALLOWAY, J. N., TOWNSEND, A. R., ERISMAN, J. W., BEKUNDA, M., CAI, Z., FRENEY, J. R., MARTINELLI, L. A., SEITZINGER, S. P. & SUTTON, M. A. 2008. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science*, 320, 889-892.
- GIARDINA, C. P., LITTON, C. M., CROW, S. E. & ASNER, G. P. 2014. Warming-Related Increases in Soil CO₂ Efflux Are Explained by Increased Below-Ground Carbon Flux. *Nature Climate Change*, 4, 822-827.
- GOLL, D., BROVKIN, V., PARIDA, B., REICK, C., KATTGE, J., REICH, P., VAN BODEGOM, P. & NIINEMETS, Ü. 2012. Nutrient Limitation Reduces Land Carbon Uptake in Simulations with a Model of Combined Carbon, Nitrogen and Phosphorus Cycling. *Biogeosciences Discussions*, 9.
- GOULDING, K., BAILEY, N., BRADBURY, N., HARGREAVES, P., HOWE, M., MURPHY, D., POULTON, P. & WILLISON, T. 1998. Nitrogen Deposition and Its Contribution to Nitrogen Cycling and Associated Soil Processes. *New Phytologist*, 139, 49-58.
- GOWER, S. T., KUCHARIK, C. J. & NORMAN, J. M. 1999. Direct and Indirect Estimation of Leaf Area Index, $\int PAR$, and Net Primary Production of Terrestrial Ecosystems. *Remote Sensing of Environment*, 70, 29-51.
- GRAHAM, P. H. & VANCE, C. P. 2003. Legumes: Importance and Constraints to Greater Use. *Plant physiology*, 131, 872-877.
- GRAY, C. M. & FIERER, N. 2012. Impacts of Nitrogen Fertilization on Volatile Organic Compound Emissions from Decomposing Plant Litter. *Global Change Biology*, 18, 739-748.
- GRUBER, N. & GALLOWAY, J. N. 2008. An Earth-System Perspective of the Global Nitrogen Cycle. *Nature*, 451, 293-296.
- GUNDALE, M. J., NILSSON, M., BANSAL, S. & JÄDERLUND, A. 2012. The Interactive Effects of Temperature and Light on Biological Nitrogen Fixation in Boreal Forests. *New Phytologist*, 194, 453-463.
- GUO, L. B. & GIFFORD, R. 2002. Soil Carbon Stocks and Land Use Change: A Meta Analysis. *Global change biology*, 8, 345-360.
- HANSON, P., EDWARDS, N., GARTEN, C. & ANDREWS, J. 2000. Separating Root and Soil Microbial Contributions to Soil Respiration: A Review of Methods and Observations. *Biogeochemistry*, 48, 115-146.
- HARDARSON, G. & ATKINS, C. 2003. Optimising Biological N₂ Fixation by Legumes in Farming Systems. *Plant and Soil*, 252, 41-54.
- HARLEY, J. 1971. Fungi in Ecosystems. *Journal of Applied Ecology*, 627-642.
- HARRISON, A. F. 1987. *Soil Organic Phosphorus: A Review of World Literature*, CAB International Wallingford, UK.
- HARTLEY, I., HOPKINS, D., SOMMERKORN, M. & WOOKEY, P. 2010. The Response of Organic Matter Mineralisation to Nutrient and Substrate Additions in Sub-Arctic Soils. *Soil Biology and Biochemistry*, 42, 92-100.

- HARTLEY, I. P. 2014. Soil Carbon: Resisting Climate Change. *Nature Climate Change*, 4, 760-761.
- HARTLEY, I. P., HEINEMEYER, A., EVANS, S. P. & INESON, P. 2007. The Effect of Soil Warming on Bulk Soil Vs. Rhizosphere Respiration. *Global Change Biology*, 13, 2654-2667.
- HARTLEY, I. P., HOPKINS, D. W., GARNETT, M. H., SOMMERKORN, M. & WOOKEY, P. A. 2008. Soil Microbial Respiration in Arctic Soil Does Not Acclimate to Temperature. *Ecology Letters*, 11, 1092-1100.
- HARTLEY, I. P. & INESON, P. 2008. Substrate Quality and the Temperature Sensitivity of Soil Organic Matter Decomposition. *Soil Biology & Biochemistry*, 40, 1567-1574.
- HAYGARTH, P., BILOTTA, G., BOL, R., BRAZIER, R., BUTLER, P., FREER, J., GIMBERT, L., GRANGER, S., KRUEGER, T. & MACLEOD, C. 2006. Processes Affecting Transfer of Sediment and Colloids, with Associated Phosphorus, from Intensively Farmed Grasslands: An Overview of Key Issues. *Hydrological Processes*, 20, 4407-4413.
- HEINEMEYER, A., HARTLEY, I. P., EVANS, S. P., DE LA FUENTE, J. A. C. & INESON, P. 2007. Forest Soil Co₂ Flux: Uncovering the Contribution and Environmental Responses of Ectomycorrhizas. *Global Change Biology*, 13, 1786-1797.
- HOBBIE, S. E. 2005. Contrasting Effects of Substrate and Fertilizer Nitrogen on the Early Stages of Litter Decomposition. *Ecosystems*, 8, 644-656.
- HOBBIE, S. E. & VITOUSEK, P. M. 2000. Nutrient Limitation of Decomposition in Hawaiian Forests. *Ecology*, 81, 1867-1877.
- HODGE, A., ROBINSON, D. & FITTER, A. 2000. Are Microorganisms More Effective Than Plants at Competing for Nitrogen? *Trends in Plant Science*, 5, 304-308.
- HÖGBERG, P., BHUPINDERPAL, S., LÖFVENIUS, M. O. & NORDGREN, A. 2009. Partitioning of Soil Respiration into Its Autotrophic and Heterotrophic Components by Means of Tree-Girdling in Old Boreal Spruce Forest. *Forest Ecology and Management*, 257, 1764-1767.
- HÖGBERG, P., NORDGREN, A., HÖGBERG, M., OTTOSSON-LÖFVENIUS, M., OLSSON, P. & LINDER, S. 2004. Fractional Contributions by Autotrophic and Heterotrophic Respiration to Soil-Surface Co₂ Efflux in Boreal Forests. *SEB experimental biology series*, 251-267.
- HÖGBERG, P. & READ, D. J. 2006. Towards a More Plant Physiological Perspective on Soil Ecology. *Trends in Ecology & Evolution*, 21, 548-554.
- HOPKINS, D. W., WAITE, I. S. & O'DONNELL, A. G. 2011. Microbial Biomass, Organic Matter Mineralization and Nitrogen in Soils from Long-Term Experimental Grassland Plots (Palace Leas Meadow Hay Plots, UK). *European Journal of Soil Science*, 62, 95-104.
- HORSWILL, P., O'SULLIVAN, O., PHOENIX, G. K., LEE, J. A. & LEAKE, J. R. 2008. Base Cation Depletion, Eutrophication and Acidification of Species-Rich Grasslands in Response to Long-Term Simulated Nitrogen Deposition. *Environmental Pollution*, 155, 336-349.
- HOUGHTON, J. T. 2001. *Climate Change 2001: The Scientific Basis*, Cambridge University Press Cambridge, UK.
- HUNGATE, B. A., DUKES, J. S., SHAW, M. R., LUO, Y. Q. & FIELD, C. B. 2003. Nitrogen and Climate Change. *Science*, 302, 1512-1513.
- IPCC 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, [Stocker, Tf; Qin, D; Plattner, Gk; Tignor, M; Allen, Sk; Boschung, J; Nauels, a; Xia, Y; Bex, V; Midgley, Pm (Eds.)]. Cambridge: Cambridge University Press.

- IPCC, A. 2007. Intergovernmental Panel on Climate Change.
- ISAAC, M. E., HARMAND, J. M. & DREVON, J. J. 2011. Growth and Nitrogen Acquisition Strategies of Acacia Senegal Seedlings under Exponential Phosphorus Additions. *Journal of Plant Physiology*, 168, 776-781.
- JANSSENS, I., DIELEMAN, W., LUYSSAERT, S., SUBKE, J., REICHSTEIN, M., CEULEMANS, R., CIAIS, P., DOLMAN, A., GRACE, J. & MATTEUCCI, G. 2010. Reduction of Forest Soil Respiration in Response to Nitrogen Deposition. *Nature Geoscience*, 3, 315-322.
- JAOUDE, A., LAGOMARSINO, A. & DE ANGELIS, P. 2011. Impacts of Nitrogen Fertilisation and Coppicing on Total and Heterotrophic Soil Co₂ Efflux in a Short Rotation Poplar Plantation. *Plant and Soil*, 339, 1, 219-230.
- JENKINSON, D. 2001. The Impact of Humans on the Nitrogen Cycle, with Focus on Temperate Arable Agriculture. *Plant and Soil*, 228, 3-15.
- JENKINSON, D., ANDREW, S., LYNCH, J., GOSS, M. & TINKER, P. 1990. The Turnover of Organic Carbon and Nitrogen in Soil [and Discussion]. *Philosophical Transactions: Biological Sciences*, 329, 361-368.
- JENSEN, E., PEOPLES, M., BODDEY, R., GRESSHOFF, P., HAUGGAARD-NIELSEN, H., ALVES, B. & MORRISON, M. 2012. Legumes for Mitigation of Climate Change and the Provision of Feedstock for Biofuels and Biorefineries. A Review. *Agronomy for Sustainable Development*, 32, 329-364.
- JOHNSON, D., LEAKE, J. R. & LEE, J. A. 1999. The Effects of Quantity and Duration of Simulated Pollutant Nitrogen Deposition on Root-Surface Phosphatase Activities in Calcareous and Acid Grasslands: A Bioassay Approach. *New Phytologist*, 141, 433-442.
- JOHNSON, D., LEAKE, J. R., LEE, J. A. & CAMPBELL, C. D. 1998. Changes in Soil Microbial Biomass and Microbial Activities in Response to 7 Years Simulated Pollutant Nitrogen Deposition on a Heathland and Two Grasslands. *Environmental Pollution*, 103, 239-250.
- JONES, M. & DONNELLY, A. 2004. Carbon Sequestration in Temperate Grassland Ecosystems and the Influence of Management, Climate and Elevated Co₂. *New Phytologist*, 164, 423-439.
- JONES, S., REES, R., KOSMAS, D., BALL, B. & SKIBA, U. 2006. Carbon Sequestration in a Temperate Grassland; Management and Climatic Controls. *Soil use and management*, 22, 132-142.
- JOOS, F., PRENTICE, I. C., SITCH, S., MEYER, R., HOOSS, G., PLATTNER, G. K., GERBER, S. & HASSELMANN, K. 2001. Global Warming Feedbacks on Terrestrial Carbon Uptake under the Intergovernmental Panel on Climate Change (Ipcc) Emission Scenarios. *Global Biogeochemical Cycles*, 15, 891-907.
- KAO, S. & LIU, K. 2000. Stable Carbon and Nitrogen Isotope Systematics in a Human-Disturbed Watershed (Lanyang-Hsi) in Taiwan and the Estimation of Biogenic Particulate Organic Carbon and Nitrogen Fluxes. *Global Biogeochemical Cycles*, 14, 189-198.
- KATTGE, J., KNORR, W., RADDATZ, T. & WIRTH, C. 2009. Quantifying Photosynthetic Capacity and Its Relationship to Leaf Nitrogen Content for Global-Scale Terrestrial Biosphere Models. *Global Change Biology*, 15, 976-991.
- KAYE, J. P. & HART, S. C. 1997. Competition for Nitrogen between Plants and Soil Microorganisms. *Trends in Ecology & Evolution*, 12, 139-143.

- KEELER, B. L., HOBBIE, S. E. & KELLOGG, L. E. 2009. Effects of Long-Term Nitrogen Addition on Microbial Enzyme Activity in Eight Forested and Grassland Sites: Implications for Litter and Soil Organic Matter Decomposition. *Ecosystems*, 12, 1-15.
- KIRSCHBAUM, M. U. F. 1995. The Temperature Dependence of Soil Organic Matter Decomposition, and the Effect of Global Warming on Soil Organic C Storage. *Soil Biology and Biochemistry*, 27, 753-760.
- KNOPS, J. M., WEDIN, D. & TILMAN, D. 2001. Biodiversity and Decomposition in Experimental Grassland Ecosystems. *Oecologia*, 126, 429-433.
- KUZYAKOV, Y. 2002. Review: Factors Affecting Rhizosphere Priming Effects. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, 165, 382-396.
- KUZYAKOV, Y. 2006. Sources of Co₂ Efflux from Soil and Review of Partitioning Methods. *Soil Biology and Biochemistry*, 38, 425-448.
- KUZYAKOV, Y. & DOMANSKI, G. 2000. Carbon Input by Plants into the Soil. Review. *Journal of Plant Nutrition and Soil Science*, 163, 421-431.
- LAIRD, D., FLEMING, P., WANG, B., HORTON, R. & KARLEN, D. 2010. Biochar Impact on Nutrient Leaching from a Midwestern Agricultural Soil. *Geoderma*, 158, 436-442.
- LAL, R. 2004. Soil Carbon Sequestration to Mitigate Climate Change. *Geoderma*, 123, 1-22.
- LAL, R. 2008. Sequestration of Atmospheric Co₂ in Global Carbon Pools. *Energy & Environmental Science*, 1, 86-100.
- LAMBERS, H., MOUGEL, C., JAILLARD, B. & HINSINGER, P. 2009. Plant-Microbe-Soil Interactions in the Rhizosphere: An Evolutionary Perspective. *Plant and Soil*, 321, 83-115.
- LARUE, T. A. & PATTERSON, T. G. 1981. How Much Nitrogen Do Legumes Fix. *Adv. Agron*, 34, 15-38.
- LAUBER, C. L., STRICKLAND, M. S., BRADFORD, M. A. & FIERER, N. 2008. The Influence of Soil Properties on the Structure of Bacterial and Fungal Communities across Land-Use Types. *Soil Biology and Biochemistry*, 40, 2407-2415.
- LE QUERE, C. 2010. Trends in the Land and Ocean Carbon Uptake. *Current Opinion in Environmental Sustainability*, 2, 219-224.
- LEDGARD, S. F. 2001. Nitrogen Cycling in Low Input Legume-Based Agriculture, with Emphasis on Legume/Grass Pastures. *Plant and Soil*, 228, 43-59.
- LEDGARD, S. F., JARVIS, S. C. & HATCH, D. J. 1998. Short-Term Nitrogen Fluxes in Grassland Soils under Different Long-Term Nitrogen Management Regimes. *Soil Biology and Biochemistry*, 30, 1233-1241.
- LEDGARD, S. F. & STEELE, K. W. 1992. Biological Nitrogen Fixation in Mixed Legume/Grass Pastures. *Plant and Soil*, 141, 137-153.
- LEE, J. A. & CAPORN, S. J. M. 1998. Ecological Effects of Atmospheric Reactive Nitrogen Deposition on Semi-Natural Terrestrial Ecosystems. *New Phytologist*, 139, 127-134.
- LI, H., FU, S., ZHAO, H. & XIA, H. 2011. Forest Soil Co₂ Fluxes as a Function of Understory Removal and N-Fixing Species Addition. *Journal of Environmental Sciences*, 23, 949-957.
- LIN, X., ZHANG, Z., WANG, S., HU, Y., XU, G., LUO, C., CHANG, X., DUAN, J., LIN, Q. & XU, B. 2011. Response of Ecosystem Respiration to Warming and Grazing During the Growing

- Seasons in the Alpine Meadow on the Tibetan Plateau. *Agricultural and Forest Meteorology*, 151, 792-802.
- LU, X., MO, J., GILLIAM, F., ZHOU, G. & FANG, Y. 2010. Effects of Experimental Nitrogen Additions on Plant Diversity in an Old-Growth Tropical Forest. *Global Change Biology*.
- LUO, Y., SU, B., CURRIE, W. S., DUKES, J. S., FINZI, A., HARTWIG, U., HUNGATE, B., MCMURTRIE, R. E., OREN, R. & PARTON, W. J. 2004. Progressive Nitrogen Limitation of Ecosystem Responses to Rising Atmospheric Carbon Dioxide. *Bioscience*, 54, 731-739.
- MACK, M. C., SCHUUR, E. A. G., BRET-HARTE, M. S., SHAVER, G. R. & CHAPIN, F. S. 2004. Ecosystem Carbon Storage in Arctic Tundra Reduced by Long-Term Nutrient Fertilization. *Nature*, 431, 440-443.
- MAGNANI, F., MENCUCCINI, M., BORGHETTI, M., BERBIGIER, P., BERNINGER, F., DELZON, S., GRELE, A., HARI, P., JARVIS, P. G., KOLARI, P., KOWALSKI, A. S., LANKREIJER, H., LAW, B. E., LINDROTH, A., LOUSTAU, D., MANCA, G., MONCRIEFF, J. B., RAYMENT, M., TEDESCHI, V., VALENTINI, R. & GRACE, J. 2007. The Human Footprint in the Carbon Cycle of Temperate and Boreal Forests. *Nature*, 447, 848-850.
- MAIA, S., OGLE, S., CERRI, C. & CERRI, C. 2010. Soil Organic Carbon Stock Change Due to Land Use Activity Along the Agricultural Frontier of the Southwestern Amazon, Brazil, between 1970 and 2002. *Global Change Biology*.
- MANZONI, S., TAYLOR, P., RICHTER, A., PORPORATO, A. & ÅGREN, G. I. 2012. Environmental and Stoichiometric Controls on Microbial Carbon-Use Efficiency in Soils. *New Phytologist*, 196, 79-91.
- MARTIN, A., MARIOTTI, A., BALESSENT, J. & LAVELLE, P. 1992. Soil Organic Matter Assimilation by a Geophagous Tropical Earthworm Based on $(\Delta)^{13}C$ Measurements. *Ecology*, 118-128.
- MASCLAUX-DAUBRESSE, C., DANIEL-VEDELE, F., DECHORGNAT, J., CHARDON, F., GAUFICHON, L. & SUZUKI, A. 2010. Nitrogen Uptake, Assimilation and Remobilization in Plants: Challenges for Sustainable and Productive Agriculture. *Annals of Botany*, 105, 1141-1157.
- MCFARLAND, J. W., WALDROP, M. P. & HAW, M. 2013. Extreme CO₂ Disturbance and the Resilience of Soil Microbial Communities. *Soil Biology and Biochemistry*, 65, 274-286.
- MEEHL, G. A., T.F. Stocker, W.D. Collins, P. Friedlingstein, A.T. Gaye, J.M. Gregory, A. Kitoh, R. Knutti, J.M. Murphy, A. Noda, S.C.B. Raper, I.G. Watterson, A.J. Weaver and Z.-C. Zhao 2007. 2007: Global Climate Projections. In: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]*, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- MEIER, C. L. & BOWMAN, W. D. 2008. Links between Plant Litter Chemistry, Species Diversity, and Below-Ground Ecosystem Function. *Proceedings of the National Academy of Sciences*, 105, 19780-19785.
- MEIR, P., COX, P. & GRACE, J. 2006. The Influence of Terrestrial Ecosystems on Climate. *Trends in Ecology & Evolution*, 21, 254-260.
- MELILLO, J. M., STEUDLER, P. A., ABER, J. D., NEWKIRK, K., LUX, H., BOWLES, F. P., CATRICALA, C., MAGILL, A., AHRENS, T. & MORRISSEAU, S. 2002. Soil Warming and Carbon-Cycle Feedbacks to the Climate System. *Science*, 298, 2173-2176.

- MITSCH, W. J., DAY, J. W., GILLIAM, J. W., GROFFMAN, P. M., HEY, D. L., RANDALL, G. W. & WANG, N. 2001. Reducing Nitrogen Loading to the Gulf of Mexico from the Mississippi River Basin: Strategies to Counter a Persistent Ecological Problem Ecotechnology—the Use of Natural Ecosystems to Solve Environmental Problems—Should Be a Part of Efforts to Shrink the Zone of Hypoxia in the Gulf of Mexico. *BioScience*, 51, 373-388.
- MORECROFT, M. D., SELLERS, E. K. & LEE, J. A. 1994. An Experimental Investigation into the Effects of Atmospheric Nitrogen Deposition on Two Semi-Natural Grasslands. *Journal of Ecology*, 82, 475-483.
- NERC 2007. Countryside Survey - England Results from 2007: Chapter 4 • Semi-Natural Grasslands: Neutral, Calcareous and Acid Grasslands.
- NILSSON, J. 1988. Critical Loads for Sulphur and Nitrogen. In: MATHY, P. (ed.) *Air Pollution and Ecosystems*. Springer Netherlands.
- NORBY, R. J., DELUCIA, E. H., GIELEN, B., CALFAPIETRA, C., GIARDINA, C. P., KING, J. S., LEDFORD, J., MCCARTHY, H. R., MOORE, D. J. P., CEULEMANS, R., DE ANGELIS, P., FINZI, A. C., KARNOSKY, D. F., KUBISKE, M. E., LUKAC, M., PREGITZER, K. S., SCARASCIA-MUGNOZZA, G. E., SCHLESINGER, W. H. & OREN, R. 2005. Forest Response to Elevated Co₂ Is Conserved across a Broad Range of Productivity. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 18052-18056.
- NORBY, R. J. & IVERSEN, C. M. 2006. Nitrogen Uptake, Distribution, Turnover, and Efficiency of Use in a Co₂-Enriched Sweetgum Forest. *Ecology*, 87, 5-14.
- NORBY, R. J., WARREN, J. M., IVERSEN, C. M., MEDLYN, B. E. & MCMURTRIE, R. E. 2010. Co₂ Enhancement of Forest Productivity Constrained by Limited Nitrogen Availability. *Proceedings of the National Academy of Sciences*, 107, 19368-19373.
- NYFELER, D., HUGUENIN-ELIE, O., SUTER, M., FROSSARD, E. & LÜSCHER, A. 2011. Grass-Legume Mixtures Can Yield More Nitrogen Than Legume Pure Stands Due to Mutual Stimulation of Nitrogen Uptake from Symbiotic and Non-Symbiotic Sources. *Agriculture, Ecosystems & Environment*, 140, 155-163.
- O'SULLIVAN, O. S., HORSWILL, P., PHOENIX, G. K., LEE, J. A. & LEAKE, J. R. 2011. Recovery of Soil Nitrogen Pools in Species-Rich Grasslands after 12 Years of Simulated Pollutant Nitrogen Deposition: A 6-Year Experimental Analysis. *Global Change Biology*, 17, 2615-2628.
- OLDROYD, G. E. D. & DIXON, R. 2014. Biotechnological Solutions to the Nitrogen Problem. *Current Opinion in Biotechnology*, 26, 19-24.
- OREN, R., ELLSWORTH, D. S., JOHNSEN, K. H., PHILLIPS, N., EWERS, B. E., MAIER, C., SCHAFER, K. V. R., MCCARTHY, H., HENDREY, G., MCNULTY, S. G. & KATUL, G. G. 2001. Soil Fertility Limits Carbon Sequestration by Forest Ecosystems in a Co₂-Enriched Atmosphere. *Nature*, 411, 469-472.
- PEUKERT, S., BOL, R., ROBERTS, W., MACLEOD, C. J. A., MURRAY, P. J., DIXON, E. R. & BRAZIER, R. E. 2012. Understanding Spatial Variability of Soil Properties: A Key Step in Establishing Field- to Farm-Scale Agro-Ecosystem Experiments. *Rapid Communications in Mass Spectrometry*, 26, 2413-2421.
- PHOENIX, G. K., BOOTH, R. E., LEAKE, J. R., READ, D. J., GRIME, J. P. & LEE, J. A. 2003a. Effects of Enhanced Nitrogen Deposition and Phosphorus Limitation on Nitrogen Budgets of Semi-Natural Grasslands. *Global Change Biology*, 9, 1309-1321.
- PHOENIX, G. K., BOOTH, R. E., LEAKE, J. R., READ, D. J., GRIME, J. P. & LEE, J. A. 2003b. Simulated Pollutant Nitrogen Deposition Increases P Demand and Enhances Root-Surface

- Phosphatase Activities of Three Plant Functional Types in a Calcareous Grassland. *New Phytologist*, 161, 279-289.
- PHOENIX, G. K., EMMETT, B. A., BRITTON, A. J., CAPORN, S. J. M., DISE, N. B., HELLIWELL, R., JONES, L., LEAKE, J. R., LEITH, I. D., SHEPPARD, L. J., SOWERBY, A., PILKINGTON, M. G., ROWE, E. C., ASHMORE, M. R. & POWER, S. A. 2012. Impacts of Atmospheric Nitrogen Deposition: Responses of Multiple Plant and Soil Parameters across Contrasting Ecosystems in Long-Term Field Experiments. *Global Change Biology*, 18, 1197-1215.
- PHOENIX, G. K., HICKS, W. K., CINDERBY, S., KUYLENSTIERNA, J. C. I., STOCK, W. D., DENTENER, F. J., GILLER, K. E., AUSTIN, A. T., LEFROY, R. D. B., GIMENO, B. S., ASHMORE, M. R. & INESON, P. 2006. Atmospheric Nitrogen Deposition in World Biodiversity Hotspots: The Need for a Greater Global Perspective in Assessing N Deposition Impacts. *Global Change Biology*, 12, 470-476.
- PHOENIX, G. K., JOHNSON, D., GRIME, J. P. & BOOTH, R. E. 2008. Sustaining Ecosystem Services in Ancient Limestone Grassland: Importance of Major Component Plants and Community Composition. *Journal of Ecology*, 96, 894-902.
- PHOENIX, G. K., LEAKE, J. R., READ, D. J., GRIME, J. P. & LEE, J. A. 2004. Accumulation of Pollutant Nitrogen in Calcareous and Acidic Grasslands: Evidence from N Flux and ¹⁵N Tracer Studies. *Water, Air, & Soil Pollution: Focus*, 4, 159-167.
- PHOENIX, G. K. & LEE, J. A. 2004. Predicting Impacts of Arctic Climate Change: Past Lessons and Future Challenges. *Ecological Research*, 19, 65-74.
- PIMENTEL, D., HEPPELRY, P., HANSON, J., DOUDS, D. & SEIDEL, R. 2005. Environmental, Energetic, and Economic Comparisons of Organic and Conventional Farming Systems. *Bioscience*, 55, 573-582.
- PLANTE, A. F., CONANT, R. T., CARLSON, J., GREENWOOD, R., SHULMAN, J. M., HADDIX, M. L. & PAUL, E. A. 2010. Decomposition Temperature Sensitivity of Isolated Soil Organic Matter Fractions. *Soil Biology & Biochemistry*, 42, 1991-1996.
- POLLEY, H., JOHNSON, H. & MAYEUX, H. 1995. Nitrogen and Water Requirements of C 3 Plants Grown at Glacial to Present Carbon Dioxide Concentrations. *Functional Ecology*, 86-96.
- POST, W. M. & KWON, K. C. 2000. Soil Carbon Sequestration and Land-Use Change: Processes and Potential. *Global Change Biology*, 6, 317-327.
- POWLSON, D. S., BROOKES, P. C., WHITMORE, A. P., GOULDING, K. W. T. & HOPKINS, D. W. 2011a. Soil Organic Matters. *European Journal of Soil Science*, 62, 1-4.
- POWLSON, D. S., WHITMORE, A. P. & GOULDING, K. W. T. 2011b. Soil Carbon Sequestration to Mitigate Climate Change: A Critical Re-Examination to Identify the True and the False. *European Journal of Soil Science*, 62, 42-55.
- PREGITZER, K. S., BURTON, A. J., ZAK, D. R. & TALHELM, A. F. 2008. Simulated Chronic Nitrogen Deposition Increases Carbon Storage in Northern Temperate Forests. *Global Change Biology*, 14, 142-153.
- PRENTICE, I. C. 2001. *Interactions of Climate Change and the Terrestrial Biosphere*.
- PRESTON, C. D., PEARMAN, D. A. & DINES, T. D. 2002. *New Atlas of the British and Irish Flora. An Atlas of the Vascular Plants of Britain, Ireland, the Isle of Man and the Channel Islands*, Oxford University Press.

- PUTTOCK, A., DUNGAIT, J. A., BOL, R., DIXON, E. R., MACLEOD, C. J. & BRAZIER, R. E. 2012. Stable Carbon Isotope Analysis of Fluvial Sediment Fluxes over Two Contrasting C4-C3 Semi-Arid Vegetation Transitions. *Rapid Communications in Mass Spectrometry*, 26, 2386-2392.
- RAICH, J. W., RUSSELL, A. E., KITAYAMA, K., PARTON, W. J. & VITOUSEK, P. M. 2006. Temperature Influences Carbon Accumulation in Moist Tropical Forests. *Ecology*, 87, 76-87.
- RASTETTER, E. B., AGREN, G. I. & SHAVER, G. R. 1997. Responses of N-Limited Ecosystems to Increased Co₂: A Balanced-Nutrition, Coupled-Element-Cycles Model. *Ecological Applications*, 7, 444-460.
- REICHSTEIN, M., SUBKE, J. A., ANGELI, A. C. & TENHUNEN, J. D. 2005. Does the Temperature Sensitivity of Decomposition of Soil Organic Matter Depend Upon Water Content, Soil Horizon, or Incubation Time? *Global Change Biology*, 11, 1754-1767.
- RUBINO, M., DUNGAIT, J., EVERSHERD, R., BERTOLINI, T., DE ANGELIS, P., D'ONOFRIO, A., LAGOMARSINO, A., LUBRITTO, C., MEROLA, A. & TERRASI, F. 2010. Carbon Input Belowground Is the Major C Flux Contributing to Leaf Litter Mass Loss: Evidences from a 13 C Labelled-Leaf Litter Experiment. *Soil Biology and Biochemistry*, 42, 1009-1016.
- RYLE, G., POWELL, C. & GORDON, A. 1979. The Respiratory Costs of Nitrogen Fixation in Soyabean, Cowpea, and White Clover I. Nitrogen Fixation and the Respiration of the Nodulated Root. *Journal of Experimental Botany*, 30, 135-144.
- SCHIMEL, D. S. 1995. Terrestrial Ecosystems and the Carbon-Cycle. *Global Change Biology*, 1, 77-91.
- SCHIMEL, D. S., HOUSE, J., HIBBARD, K., BOUSQUET, P., CIAIS, P., PEYLIN, P., BRASWELL, B. H., APPS, M. J., BAKER, D. & BONDEAU, A. 2001. Recent Patterns and Mechanisms of Carbon Exchange by Terrestrial Ecosystems. *Nature*.
- SCHLESINGER, W. H. 1997. *Biogeochemistry: An Analysis of Global Change*, Academic press.
- SCHLESINGER, W. H. & ANDREWS, J. A. 2000. Soil Respiration and the Global Carbon Cycle. *Biogeochemistry*, 48, 7-20.
- SCHMIDT, M. W., TORN, M. S., ABIVEN, S., DITTMAR, T., GUGGENBERGER, G., JANSSENS, I. A., KLEBER, M., KÖGEL-KNABNER, I., LEHMANN, J. & MANNING, D. A. 2011. Persistence of Soil Organic Matter as an Ecosystem Property. *Nature*, 478, 49-56.
- SCHOFFELEN, P. F., WESTERTERP, K. R., SARIS, W. H. & TEN HOOR, F. 1997. A Dual-Respiration Chamber System with Automated Calibration. *Journal of Applied Physiology*, 83, 2064-2072.
- SHAVER, G. R., BILLINGS, W. D., CHAPIN, F. S., GIBLIN, A. E., NADELHOFFER, K. J., OECHEL, W. C. & RASTETTER, E. B. 1992. Global Change and the Carbon Balance of Arctic Ecosystems. *Bioscience*, 42, 433-441.
- SHEN, J., YUAN, L., ZHANG, J., LI, H., BAI, Z., CHEN, X., ZHANG, W. & ZHANG, F. 2011. Phosphorus Dynamics: From Soil to Plant. *Plant Physiology*, 156, 997-1005.
- SHIBATA, H., BRANQUINHO, C., MCDOWELL, W., MITCHELL, M., MONTEITH, D., TANG, J., ARVOLA, L., CRUZ, C., CUSACK, D., HALADA, L., KOPÁČEK, J., MÁGUAS, C., SAJIDU, S., SCHUBERT, H., TOKUCHI, N. & ZÁHORA, J. 2014. Consequence of Altered Nitrogen Cycles in the Coupled Human and Ecological System under Changing Climate: The Need for Long-Term and Site-Based Research. *AMBIO*, 1-16.

- SILLEN, W. & DIELEMAN, W. 2012. Effects of Elevated Co₂ and N Fertilization on Plant and Soil Carbon Pools of Managed Grasslands: A Meta-Analysis.
- SISTLA, S. A. & SCHIMEL, J. P. 2012. Stoichiometric Flexibility as a Regulator of Carbon and Nutrient Cycling in Terrestrial Ecosystems under Change. *New Phytologist*.
- SIX, J., CONANT, R. T., PAUL, E. A. & PAUSTIAN, K. 2002. Stabilization Mechanisms of Soil Organic Matter: Implications for C-Saturation of Soils. *Plant and Soil*, 241, 155-176.
- SIX, J. P., ELLIOTT, K. & COMBRINK, E. 2000. Soil Structure and Organic Matter. *Soil Science Society of America Journal*, 64, 681–689.
- SMITH, S. E. & READ, D. J. 2010. *Mycorrhizal Symbiosis*, Academic press.
- SOLOMON, S., QIN, D., MANNING, M., CHEN, Z., MARQUIS, M., AVERYT, K., TIGNOR, M. & MILLER, H. 2007. Ipcc, 2007: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. New York: Cambridge University Press.
- SORENSEN, P. L., LETT, S. & MICHELSEN, A. 2012. Moss-Specific Changes in Nitrogen Fixation Following Two Decades of Warming, Shading, and Fertilizer Addition. *Plant Ecology*, 213, 695-706.
- SOUSSANA, J., TALLEC, T. & BLANFORT, V. 2010. Mitigating the Greenhouse Gas Balance of Ruminant Production Systems through Carbon Sequestration in Grasslands. *animal*, 4, 334-350.
- SOUSSANA, J. F., LOISEAU, P., VUICHARD, N., CESCHIA, E., BALESSENT, J., CHEVALLIER, T. & ARROUAYS, D. 2004. Carbon Cycling and Sequestration Opportunities in Temperate Grasslands. *Soil use and management*, 20, 219-230.
- STADDON, P., HEINEMEYER, A. & FITTER, A. 2002. Mycorrhizas and Global Environmental Change: Research at Different Scales. *Diversity and Integration in Mycorrhizas*. Springer.
- STEVENS, C. J., DISE, N. B., MOUNTFORD, J. O. & GOWING, D. J. 2004. Impact of Nitrogen Deposition on the Species Richness of Grasslands. *Science*, 303, 1876-1879.
- STEVENSON, B., KELLY, E., MCDONALD, E. & BUSACCA, A. 2005. The Stable Carbon Isotope Composition of Soil Organic Carbon and Pedogenic Carbonates Along a Bioclimatic Gradient in the Palouse Region, Washington State, USA. *Geoderma*, 124, 37-47.
- STEWART, C. E., PAUSTIAN, K., CONANT, R. T., PLANTE, A. F. & SIX, J. 2009. Soil Carbon Saturation: Implications for Measurable Carbon Pool Dynamics in Long-Term Incubations. *Soil Biology & Biochemistry*, 41, 357-366.
- STOCKER, T. F., QIN, D., PLATTNER, G.-K., TIGNOR, M., ALLEN, S. K., BOSCHUNG, J., NAUELS, A., XIA, Y., BEX, V. & MIDGLEY, P. M. 2013. Climate Change 2013. The Physical Science Basis. Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change-Abstract for Decision-Makers. Groupe d'experts intergouvernemental sur l'évolution du climat/Intergovernmental Panel on Climate Change-IPCC, C/O World Meteorological Organization, 7bis Avenue de la Paix, CP 2300 CH-1211 Geneva 2 (Switzerland).
- STREET, L. E., SHAVER, G. R., WILLIAMS, M. & VAN WIJK, M. T. 2007. What Is the Relationship between Changes in Canopy Leaf Area and Changes in Photosynthetic Co₂ Flux in Arctic Ecosystems? *Journal of Ecology*, 95, 139-150.
- SUTTON, M. A., BLEEKER, A., HOWARD, C., BEKUNDA, M., GRIZZETTI, B., VRIES, W. d., VAN GRINSVEN, H., ABROL, Y., ADHYA, T. & BILLEN, G. 2013. *Our Nutrient World: The*

Challenge to Produce More Food and Energy with Less Pollution, Centre for Ecology and Hydrology (CEH).

- SUTTON, M. A., HOWARD, C. M., ERISMAN, J. W., BILLEN, G., BLEEKER, A., GRENNFELT, P., VAN GRINSVEN, H. & GRIZZETTI, B. 2011a. *The European Nitrogen Assessment: Sources, Effects and Policy Perspectives*, Cambridge University Press.
- SUTTON, M. A., OENEMA, O., ERISMAN, J. W., LEIP, A., VAN GRINSVEN, H. & WINIWARTER, W. 2011b. Too Much of a Good Thing. *Nature*, 472, 159-161.
- TANG, C., UNKOVICH, M. & BOWDEN, J. 1999. Factors Affecting Soil Acidification under Legumes. Iii. Acid Production by N₂-Fixing Legumes as Influenced by Nitrate Supply. *New Phytologist*, 143, 513-521.
- THORNTON, P. E., DONEY, S. C., LINDSAY, K., MOORE, J. K., MAHOWALD, N., RANDERSON, J. T., FUNG, I., LAMARQUE, J. F., FEDDEMA, J. J. & LEE, Y. H. 2009. Carbon-Nitrogen Interactions Regulate Climate-Carbon Cycle Feedbacks: Results from an Atmosphere-Ocean General Circulation Model. *Biogeosciences*, 6, 2099-2120.
- TORN, M. S., VITOUSEK, P. M. & TRUMBORE, S. E. 2005. The Influence of Nutrient Availability on Soil Organic Matter Turnover Estimated by Incubations and Radiocarbon Modeling. *Ecosystems*, 8, 352-372.
- TRESEDER, K. K. 2004. A Meta-Analysis of Mycorrhizal Responses to Nitrogen, Phosphorus, and Atmospheric Co₂ in Field Studies. *New Phytologist*, 164, 347-355.
- UCHIDA, Y., HUNT, J. E., BARBOUR, M. M., CLOUGH, T. J., KELLIHER, F. M. & SHERLOCK, R. R. 2010. Soil Properties and Presence of Plants Affect the Temperature Sensitivity of Carbon Dioxide Production by Soils. *Plant and soil*, 337, 375-387.
- UNKOVICH, M., HERRIDGE, D., PEOPLES, M., CADISCH, G., BODDEY, B., GILLER, K., ALVES, B. & CHALK, P. 2008. *Measuring Plant-Associated Nitrogen Fixation in Agricultural Systems*, Australian Centre for International Agricultural Research (ACIAR).
- VAN DER HEIJDEN, M. G. A., BOLLER, T., WIEMKEN, A. & SANDERS, I. R. 1998. Different Arbuscular Mycorrhizal Fungal Species Are Potential Determinants of Plant Community Structure. *Ecology*, 79, 2082-2091.
- VAN GROENIGEN, K. J., SIX, J., HUNGATE, B. A., DE GRAAFF, M. A., VAN BREEMEN, N. & VAN KESSEL, C. Element Interactions Limit Soil Carbon Storage. Proceedings of the National Academy of Science of the United States of America, 2006. National Acad Sciences.
- VITOUSEK, P. M., ABER, J. D., HOWARTH, R. W., LIKENS, G. E., MATSON, P. A., SCHINDLER, D. W., SCHLESINGER, W. H. & TILMAN, D. G. 1997. Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. *Ecological Applications*, 7, 737-750.
- VITOUSEK, P. M., CASSMAN, K., CLEVELAND, C., CREWS, T., FIELD, C. B., GRIMM, N. B., HOWARTH, R. W., MARINO, R., MARTINELLI, L. & RASTETTER, E. B. 2002a. Towards an Ecological Understanding of Biological Nitrogen Fixation. *Biogeochemistry*, 57, 1-45.
- VITOUSEK, P. M., HÄTTENSCHWILER, S., OLANDER, L. & ALLISON, S. 2002b. Nitrogen and Nature. *AMBIO: A Journal of the Human Environment*, 31, 97-101.
- VITOUSEK, P. M., HEDIN, L. O., MATSON, P. A., FOWNES, J. H. & NEFF, J. 1998. *Within-System Element Cycles, Input-Output Budgets, and Nutrient Limitation*, New York, Springer.

- VITOUSEK, P. M., PORDER, S., HOULTON, B. Z. & CHADWICK, O. A. 2010. Terrestrial Phosphorus Limitation: Mechanisms, Implications, and Nitrogen-Phosphorus Interactions. *Ecological Applications*, 20, 5-15.
- VLEESHOUWERS, L. & VERHAGEN, A. 2002. Carbon Emission and Sequestration by Agricultural Land Use: A Model Study for Europe. *Global change biology*, 8, 519-530.
- WALDROP, M. P., ZAK, D. R., SINSABAUGH, R. L., GALLO, M. & LAUBER, C. 2004. Nitrogen Deposition Modifies Soil Carbon Storage through Changes in Microbial Enzymatic Activity. *Ecological Applications*, 14, 1172-1177.
- WATSON, C. A., ATKINSON, D., GOSLING, P., JACKSON, L. R. & RAYNS, F. W. 2002. Managing Soil Fertility in Organic Farming Systems. *Soil use and management*, 18, 239-247.
- WEINTRAUB, M. N. & SCHIMEL, J. P. 2003. Interactions between Carbon and Nitrogen Mineralization and Soil Organic Matter Chemistry in Arctic Tundra Soils. *Ecosystems*, 6, 129-143.
- WILMKING, M., HARDEN, J. & TAPE, K. 2006. Effect of Tree Line Advance on Carbon Storage in Nw Alaska. *Journal of Geophysical Research: Biogeosciences (2005–2012)*, 111.
- WOFSY, S. C. 2001. "Where Has All the Carbon Gone?" (Vol 292, Pg 2261, 2001). *Science*, 294, 2480-2480.
- ZAEHLE, S., FRIEDLINGSTEIN, P. & FRIEND, A. D. 2010. Terrestrial Nitrogen Feedbacks May Accelerate Future Climate Change. *Geophysical Research Letters*, 37, 5.
- ZENG, D.-H., LI, L.-J., FAHEY, T. J., YU, Z.-Y., FAN, Z.-P. & CHEN, F.-S. 2010. Effects of Nitrogen Addition on Vegetation and Ecosystem Carbon in a Semi-Arid Grassland. *Biogeochemistry*, 98, 185-193.
- ZHAO, M. & RUNNING, S. W. 2010. Drought-Induced Reduction in Global Terrestrial Net Primary Production from 2000 through 2009. *science*, 329, 940-943.
- ZHU, Y.-G. & MILLER, M. R. 2003. Carbon Cycling by Arbuscular Mycorrhizal Fungi in Soil–Plant Systems. *Trends in plant science*, 8, 407-409.

